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UREA CLEARANCE IN NORMAL DOGS[1]

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Recently Ralli, Brown and Pariente (1) and Jolliffe and Smith (2) (3) published studies dealing with "urea clearance" in the dog, similar to that carried out on man by Möller, McIntosh and Van Slyke (4) who developed this test for renal function in man.

The study of Ralli, Brown and Pariente (1) was made on dogs that excreted relatively small amounts of urine, while that of Jolliffe and Smith (2) (3) was made on dogs that excreted much larger amounts during the experimental period. Therefore, in order to determine the "augmentation limit," Jolliffe and Smith were obliged to combine their results with those of Ralli, Brown and Pariente (1).

At the time the present investigation was begun there were no publications on urea clearance in dogs. In a study on nephropathic dogs, it was considered desirable to add this test of renal function to the others used. Therefore, as a basis for comparison, the determination of urea clearance in normal dogs was first undertaken. The conditions and method of these experiments were somewhat different from those of the investigators mentioned above and the number of observations made on one series of animals much larger. Since small and large minute-volumes of urinary excretion occurred in all of the dogs, the augmentation limit could be determined on the one series of animals. For these reasons it was decided to publish these data which are very useful to us and which may prove of value to those interested in this subject.

EXPERIMENTS. Adult female dogs were used. They were of various mixed breeds, and showed no signs of renal disease as judged by the results of the examination of urine and blood. The diet consisted of about 1½ pounds of a cooked mixture of meat, bone, cabbage, oatmeal, rice and pota-

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TABLE 1

Dog 6

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DOG LENGTH	DATE	WEIGHT	SURFACE AREA (S. A.)	VOLUME OF URINE PER MINUTE (V)	$\sqrt{V}$	UREA NITROGEN IN URINE (U)	UREA NITROGEN IN BLOOD (B)	MAXIMUM CLEARANCE $\frac{UV}{B} = C_m$	"STANDARD CLEARANCE" $\frac{U\sqrt{V}}{B} = C_s$	CLEARANCE, $\frac{0.65U\sqrt{V}}{B} = C = *$	MAXIMUM CLEARANCE PER SQ. METER $\frac{UV}{B\,(S.A.)} = C_m/S.A.$	"STANDARD CLEARANCE" PER SQ. METER $\frac{U\sqrt{V}}{B\,(S.A.)} = C_s/S.A.$	CLEARANCE* PER SQ. METER $\frac{0.65U\sqrt{V}}{B\,(S.A.)} = C/S.A.$	AMOUNT OF WATER ADMINISTERED
cm.	*1931*	*kgm.*	*sq.m.*	*cc.*	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*							*cc.*
85	4/20	18.6	0.72	0.25	0.50	1,270	9.2	34.5	69.0	44.9	47.9	95.8	62.2	
				0.15	0.39	1,758		28.6	73.3	47.7	39.7	101.8	66.1	
	4/21	18.4	0.71	0.07	0.27	2,014	7.7	18.3	67.8	44.1	25.8	95.5	62.0	
				0.06	0.24	2,150		16.8	69.8	45.4	23.7	98.3	63.9	
	4/22	18.7	0.72	0.21	0.46	1,144	9.4	25.6	55.7	36.2	35.6	77.4	50.3	
				0.18	0.42	1,173		22.5	53.6	34.8	31.3	74.5	48.4	
	4/25	18.0	0.71	0.08	0.28	2,055	6.5	25.3	90.4	58.7	35.6	127.2	82.7	
				0.06	0.24	2,137		19.7	82.2	53.4	27.7	115.7	75.2	
	4/28	18.4	0.71	0.28	0.53	1,075	9.9	30.4	57.4	37.3	42.8	80.9	52.6	184
				0.13	0.36	2,095		27.5	76.4	49.6	38.7	107.5	69.8	
				0.13	0.36	2,150	8.7	32.1	89.2	58.0	45.2	125.5	81.6	
				0.12	0.35	2,110		29.1	83.1	54.0	41.0	117.0	76.0	
	5/1	18.2	0.71	2.10	1.45	140	5.2	56.5		56.5	79.6		79.6	365
				1.50	1.22	149		43.0		43.0	60.6		60.6	
				0.58	0.76	220	5.8	22.0		22.0	31.0		31.0	
				0.17	0.41	570		16.7	40.7	26.4	23.5	57.3	34.0	
	5/7	19.4	0.73	4.87	2.21	79	8.2	46.9		46.9	64.3		64.3	485
				2.38	1.54	112		32.5		32.5	44.5		44.5	
				0.38	0.62	510	7.2	27.1	43.7	28.4	37.1	59.9	38.9	
				0.18	0.42	799		20.1	47.9	31.1	27.5	65.6	42.6	
	5/19	20.2	0.74	3.30	1.82	75	5.3	46.7		46.7	63.1		63.1	505
				3.07	1.75	65		37.7		37.7	51.0		51.0	
				3.25	1.80	65	5.0	42.3		42.3	57.2		57.2	
				4.06	2.02	79		64.1		64.1	86.6		86.6	
				1.63	1.28	79	4.1	31.4		31.4	42.4		42.4	
				0.49	0.70	219		26.2		26.2	35.4		35.4	
	12/19	23.0	0.78	0.25	0.50	3,540	33.4	26.5	53.0	34.4	34.0	67.9	44.1	
				0.24	0.49	4,070		29.3	59.8	38.9	37.6	76.7	49.9	

toes. On Sundays, dog biscuit was the only food given. Three times a week, half a pound of fresh, lean, ground beef was given, in addition, to every dog. Most of the animals gained some weight during the period of observation (see table 1, column 3). A standardized method was adopted for the conduct of the test.

Method of test. At 6 p.m. on the night before the test all food was removed but the animal was allowed to have water until just before the beginning of the test in the morning. Water was then removed and the dog was not permitted to drink during the test period. The test was carried out without the aid of anesthesia or narcotics and no difficulty was experienced in carrying out any of the necessary procedures. The bladder was first emptied by catheterization and this procedure was repeated at intervals of one hour for two or more hours. Time was counted from the moment the first catheterization was completed and subsequent catheterization was always begun a sufficient time before the end of each hour to insure an empty bladder at the right moment. Between 5 and 10 minutes before the catheterization for the first hour specimen of urine, blood was withdrawn by syringe from the external saphenous vein. Whenever the test was continued for four or six hours, samples of blood were again withdrawn at the end of the third and fifth hours. Four hour and six hour tests were carried out only when water was administered by stomach tube at the beginning of the experiment in order to increase the output of urine. When this was done, the water was always administered one-half hour before the first emptying of the bladder. The output of urine per minute ("V," column 5, table 1) was calculated from the measured output per hour. The urea nitrogen in the blood and urine was determined by the Van Slyke-Cullen method (5).

Urea clearance was calculated separately for every hour of the test. The determination of urea nitrogen in the one sample of blood taken in every two-hour period (column 8, table 1) served for the calculation of urea clearances for the two successive hours. Maximum clearance (C_m) (column 9, table 1) and so-called standard clearance (C_s) (column 10, table 1) with and without correction for surface area were calculated. Since high and

* Under the heading of "Clearance" (C) in column 11 are included the values of maximum clearance (C_m) for volumes of urinary excretion (V, column 5) above 0.42 cc. per minute, and the values of a standard clearance calculated by the formula $\frac{U\sqrt{V_s V}}{B\ (S.\ A.)}$ for all volumes of urinary excretion up to and including 0.42 cc. per minute, where V_s, the standard volume of reference, is taken at the augmentation limit, namely, 0.42 cc. per minute. For $V_s = 0.42$ cc., $\sqrt{V_s} = 0.65$ cc., and therefore the formula for "C" becomes $\frac{0.65\ U\sqrt{V}}{B}$. In column 14 the "Clearances" per square meter $\frac{C}{S.A.}$ are listed.

low rates of urinary flow (V), (column 5, table 1) were obtained in the same dogs our data afforded an opportunity to determine the augmentation limit in one series of animals. For dogs 7, 12, 14, 17, 18 and 22, it was not possible to correct for surface area because the length of the animals had not been determined. However, their maximum and standard clearances, without correction for surface area, afforded more data for the determination of the augmentation limit, the value of which is not affected by this correc-

TABLE 2

DOG NO.	NUMBER OF OBSERVATIONS	MEAN C_m	MEAN C_s	MEAN C	MEAN $\frac{C_m}{S.A.}$	MEAN $\frac{C_s}{S.A.}$	MEAN $\frac{C}{S.A.}$
1	22	46.3	60.8	42.9	64.6	88.6	61.1
2	26	34.4	46.4	31.8	47.6	63.9	43.9
3	24	36.2	48.1	33.3	49.6	66.6	46.1
4	20	34.2	47.7	32.8	42.4	60.7	40.5
5	22	35.7	46.3	32.9	44.6	57.1	40.8
6	28	40.8	65.4	41.8	55.9	90.8	57.7
8	10	42.0	46.7	33.9	71.2	79.0	57.3
9	10	25.7	46.6	28.4	42.8	77.6	47.4
10	8	29.4	43.5	28.8	42.6	63.5	41.9
11	11	41.9	80.6	49.5	57.4	105.6	65.6
13	10	20.1	48.2	29.1	37.3	81.5	49.8
15	8	34.0	50.5	30.0	51.6	77.0	50.8
16	10	39.4	55.8	37.2	64.7	91.0	60.3
21	6	55.4	113.4	64.6	76.3	159.9	90.1
25	6	33.8	55.8	35.4	56.3	94.1	59.5
39	8	41.9	75.8	46.6	63.5	113.9	70.1
40	5	48.4	56.1	38.8	73.4	108.4	71.1
7	8	26.7	31.1	23.4			
12	10	22.9	45.4	26.2			
14	8	31.2	52.8	33.2			
17	8	39.9	65.5	41.6			
18	10	41.0	55.1	36.8			
22	6	35.2	51.9	33.9			
Mean values for all the animals		36.6±8.1	55.0±12.4	36.0±8.3	53.1±11.4	80.9±18.7	52.7±12.6

tion, as used in this study. The augmentation limit, therefore, was determined from the average values of C_m and C_s for all the dogs.

On account of lack of space, the complete data for only one dog are given, as an illustration, in table 1. Table 2 is a summary in which are given the mean values of the determinations for individual dogs and the mean values for the 23 dogs.

The mean value of C_m, for values of V above 0.42 cc. per minute for the 23 dogs (111 observations) was 36.6 cc. ±8.1.

The mean value of C_s, for values of V up to and including 0.42 cc. per minute, for the 23 dogs (173 observations) was 55.0 cc. ±12.4.

From these two mean values the volume of the augmentation limit can be calculated, and was found to be 0.43 cc. For 17 of the 23 dogs studied the values of C_m and C_s per square meter of surface area $\frac{(C_m}{(SA)}$ and $\frac{C_s)}{(SA)}$ were calculated. This made the clearances in different dogs more comparable since the animals varied considerably in weight and length. Surface area (S.A.) was determined by the following formula of Cowgill and Drabkin (6).

S.A. = $4.381\ W^{0.425} \times L^{0.725}$

S.A. = Surface area in square meters.

W. = Weight in grams.

L. = Length in centimeters.

(In table 1, W and L appear as kilograms and meters respectively.)

The mean value of $\frac{C_m}{S.A.}$ (93 observations) was 53.1 cc. ± 11.4.

The mean value of $\frac{C_s}{S.A.}$ (141 observations) was 80.9 cc. ±18.7.

The augmentation limit was 0.42 cc.

The augmentation limit for the smaller number of dogs was almost the same as the value obtained for all of the dogs, without correction for surface area.

Chart 1 illustrates graphically the values of $\frac{C_m}{(S.A.)}$ for the 17 dogs of known surface area and gives the position of the augmentation limit.

In order to be able to compare directly the values for maximum and standard clearances, previous investigators have calculated the percentage value of all of the observations, taking the mean C_m and mean C_s each as 100 per cent. In this study, to accomplish the same purpose, a method of calculation was used suggested to us by Dr. R. Dominguez, Director of Laboratories, St. Luke's Hospital, Cleveland. This method consists in the calculation of a standard clearance for all minute volumes of urine below the augmentation limit, by using the value of the augmentation limit itself, namely, 0.42 cc. per minute, instead of 1 cc. per minute, as the standard volume of reference.

Möller, McIntosh and Van Slyke (4) have shown that

$$C_s = C_m \cdot \sqrt{\frac{V_s}{V}},$$

where V_s is the standard volume of reference, that is,

$$C_s = \frac{UV}{B} \cdot \sqrt{\frac{V_s}{V}} = U\frac{\sqrt{V \cdot V_s}}{B}$$

and when $V_s = 0.42$ cc. (the augmentation limit), then $\sqrt{V_s} = 0.65$ and $C_s = \frac{0.65\ U\sqrt{V}}{B}$, or $\frac{C_s}{S.A.} = \frac{0.65\ U\sqrt{V}}{B\ (S.A.)}$. This method was mentioned but not favored by Möller (7), in the case of human beings, because he considered the variation of the individual augmentation limits too great to justify this procedure. In dogs, the variation of individual augmentation limits is also great. However, the choice of this value as the standard volume of reference, in addition to making standard and maximum clearances directly comparable is further justified by the fact that this volume (0.42 cc. per minute) is a natural standard for the dog, since it is close to the average rate of urinary excretion per minute of the adult animal. In

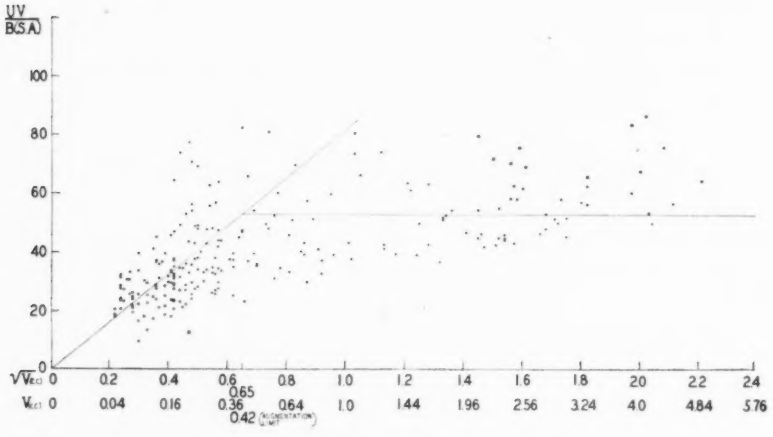

Chart 1

the 23 dogs (44 determinations) the range of excretion in 24 hours was 150 cc. to 1080 cc., with a mean value of 489 cc., which is 0.34 cc. per minute.

With all of the standard clearance values referred to the augmentation limit volume as the standard volume of reference, maximum and standard clearance values can be put in the same column and the values can then be termed collectively *the CLEARANCE* (C) (table 1, column 11). The mean value of (C) for the 23 dogs (284 observations) was 36.0 cc. ± 8.3, which is practically the same as the value 36.6 cc. ± 8.2 of C_m. The mean value of $\frac{C}{(S.A.)}$ for 17 dogs of known surface area, (234 observations) was 52.7 cc. ±12.6, per square meter, which is practically the same as that of $\frac{C_m}{S.A.}$, 53.1 cc. ± 11.4. The close correspondence of these values is to be expected

from the nature of the calculations, if the augmentation limit is a good choice as the standard volume of reference.

SUMMARY

In 23 normal dogs urea clearance and augmentation limit were determined. The mean value of the so-called standard clearances, with 1 cc. per minute taken as the standard volume of reference, and uncorrected for surface area, was 55.0 cc. ± 12.4 and, corrected for surface area, 80.9 cc. ± 18.7 per square meter of body surface. The mean value of the maximum clearances, uncorrected for surface area, was 36.6 cc. ± 8.1 and, corrected for surface area, 53.1 cc. ± 11.4 per square meter. The "augmentation limit," as determined by the urea clearances of 23 dogs (284 observations) uncorrected for surface area, was 0.43 cc. When determined by the urea clearances of only 17 dogs (234 observations), corrected for surface area, the augmentation limit was 0.42 cc. It is suggested that for the purpose of direct comparison of urea clearances at all values of V (column 5, table 1), and to avoid the so-called standard clearance, the value of the augmentation limit (0.42 cc.) should be used as the standard volume of reference, for a value is thus obtained which is truly a clearance. This makes it possible to include all values under the one term "urea clearance" without reference to "standard" and maximum clearances. In 23 dogs the mean value of the "urea clearance" (C) calculated according to this method was 36.0 cc. ± 8.3. In 17 dogs, of known surface area, $\frac{C}{(S.A.)}$ was 52.7 cc. ± 12.6 per square meter. These values are practically identical with the mean values of the maximum clearances (C_m) 36.6 cc. ± 8.1 and $\frac{C_m}{(S.A.)}$ 53.1 cc. ± 11.4 per square meter, respectively, as is to be expected when the augmentation limit is used as the standard volume of reference.

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AN EXPERIMENTAL STUDY OF "THE CONSTITUTIONAL FACTOR" IN THE ETIOLOGY OF RICKETS

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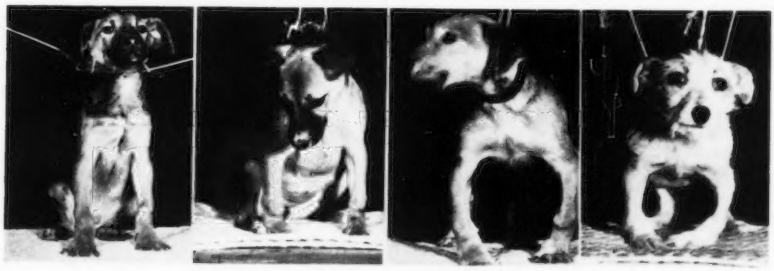
It has long been felt that, in addition to the hygienic and dietetic factors which undoubtedly play the important rôle in the etiology of rickets, some undefined and more subtle factor is of significance in the development of this disorder. For want of a more precise designation, this generally has been termed the constitutional factor. At various times, one of us has commented on this vague but nevertheless definite causative influence. In 1928, it was observed that "differences in the percentage of ash in the skeleton at birth and in the rate of growth in the early months of post-natal life are undoubtedly of importance, but in all probability deeper biologic influences of which we have no knowledge also play a rôle" (1). Somewhat later the subject was referred to in the following words: "There is some important factor in connection with the development of rickets which we have not yet fathomed and we have not yet taken sufficiently into account. Some have termed this the constitutional factor" (2). In regard to the negro infant, which is notably more susceptible to rickets than the white infant, it was felt that "in addition to this well recognized factor (pigment of the skin) the negro may possess an inherent racial tendency to rickets—a tendency perhaps shared by other races" (3). Although the reality of a constitutional disposition has been recognized, the subject has never been approached or studied from the experimental standpoint.

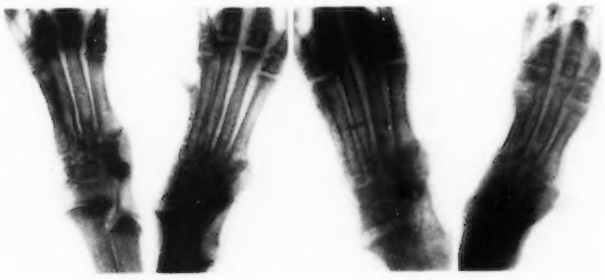
Recently the opportunity was presented of demonstrating differences in the development of rickets among individuals of a litter of puppies which had been reared under the same conditions in regard to diet and general surroundings. These four puppies were of mongrel breed, two being females and two males. It may be noted from the accompanying photographs that two were predominantly of one type, a short-haired breed, whereas the other two were of a long-haired terrier type. This distinction was evident at a glance, although there was a gradual gradation from one type to the other. Immediately after weaning, these four puppies were kept in separate cages in one room and fed the Mellanby rickets-producing diet, which consists of skim milk, cereal, cottonseed oil, sodium chloride and

sufficient tomato juice daily to prevent scurvy; it is a low calcium, high phosphorus ration. The control animal (A) which has a normal appearance both in the photograph and radiograph, received also a supplement of viosterol. The feeding experiment was carried out from November to March, a period of almost five months. The accompanying photographs show that the dog which received viosterol appeared normal with strong



A B C D

Fig. 1. Four mongrel puppies of the same litter, ranging from short-haired to long-haired type. Development and intensity of rachitic lesions at the epiphyses and bowing according to preponderance of long-haired type.



A B C D

Fig. 2. Radiographs of the puppies in preceding figure; showing same individual distribution and intensity of rickets. Note increasing width of carpal and metacarpal junctions.

straight legs, that the other short haired puppy (B) had mild rickets as evidenced by bowing of the legs and enlargement of the epiphyses at the ankle joints, that the third, somewhat shaggy puppy (C), had marked bowing of the legs, and that the legs of the fourth animal (D) were so bowed as to prevent her from standing unassisted. The radiographs of the ankles of the forelegs corroborated these appearances, showing normal

joints in the first puppy, moderate rickets in the two succeeding puppies, and marked rickets in the fourth animal.

The accompanying table gives the concentration of the calcium and inorganic phosphorus of the serum at the mid-period and at the end of the experiment. It will be noted that, whereas the serum was normal in the animal which received viosterol, it showed a definite and marked decrease of calcium in the three other animals; in the puppy with the most marked bowing of the legs, there was not only the lowest concentration of calcium, but likewise the lowest percentage of inorganic phosphorus.

As is well known, growth is an important factor in the development of rickets, the greater the growth the more marked the tendency to the development of rickets in infants and in animals. In fact, lack of growth works strongly against the induction of this disorder. In the accompanying table it may be noted that the puppy which developed rickets in great-

TABLE 1

Concentration of calcium and of inorganic phosphorus in serum of the four puppies

PUPPY	WEIGHT		SERUM CALCIUM		SERUM PHOSPHORUS	
	At onset	At end	Mid-period	At end	Mid-period	At end
	kgm.	*kgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A ♂	2 3	6.0	10.0	11.1	8 2	8 3
B ♀	1 85	5 7	7.1	6.1	6.3	5 8
C ♂	2.9	5 84	6 6	7.0	6 6	5 8
D ♀	2 14	3 71	5.7	5 5	6 0	5 6

est intensity made the least gain during the three months period, so that growth can be excluded as an inciting factor in this experiment.

A result such as the foregoing seems to indicate that one breed of dog is more susceptible to rickets than another. In other words it can be demonstrated that there is a definite constitutional tendency to rickets, quite apart from diet, hygiene and growth. This must be evident to those who have studied clinical rickets. Among infants brought up in the same institution and receiving the same diet and the same care, some develop normally whereas others develop moderate or even a marked degree of rickets. A similar distinction, from the point of view of constitution, holds for other disorders of the bones and is a factor in the varying susceptibility of the teeth to caries.[1] The method of approach which we have outlined seems well suited not only for studies of the constitutional factor in rickets but of other disturbances in the development of the bony skeleton.

[1] The teeth of these puppies are being subjected to histologic study by investigators of the Committee on Dental Caries, supported by the Commonwealth Fund.

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THE CHEMICAL MEDIATION OF AUTONOMIC NERVOUS IMPULSES AS EVIDENCED BY SUMMATION OF RESPONSES

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Recent studies have shown that the stimulation of autonomic nerves sets free chemical substances which may pass into the blood stream and show a sympathomimetic or a parasympathomimetic action on other structures (see Cannon, 1931, for bibliography). The studies on the sympathetic give support to the idea expressed by Elliott (1904), that sympathetic impulses liberate adrenin locally and that it is this adrenin which conditions the response of the effector.

It has been recently shown that the curves of the responses to varying doses of adrenin are hyperbolas and that the mode of action of the hormone is therefore probably chemical (Rosenblueth, 1932). From the all-or-none character of nervous impulses it follows that the amounts of mediator produced must be proportional to the frequency of the impulses, and, if the hypothesis of a chemical mediation be correct, the curves of the responses to varying frequencies up to a limit which will involve the refractory period of the nerve or the end organ should be identical with those obtained by varying doses of adrenin.

The present paper is a quantitative study of the responses of different structures innervated by the autonomic nervous system to varying frequencies of stimuli.

METHODS. Cats were used for all experiments except some on the submaxillary gland, in which dogs were found more suitable.

Dial anesthesia (0.6 to 0.9 cc. per kilo intraperitoneally or by stomach tube) was utilized, save in the observations on the actions of the splanchnic on the intestine and the accelerators on the heart. Since anesthetics are likely to modify the responses of these organs, the animals were swiftly pithed through the temporal bone and then used without anesthesia (see Cannon and Britton, 1925).

The curves obtained from the data furnished by the experiments were tested by the usual methods. Semi-logarithmic and logarithmic plottings eliminated exponentials and parabolas. After adjustment to adequate

[1] Fellow of the John Simon Guggenheim Memorial Foundation.

scales, symmetry of the curve to the bisector of the right angle formed by the two asymptotes was examined by transparence after folding the paper along the line. The curves were invariably found to be symmetrical to this line, as befits a hyperbola. Parabolas and exponentials would not show this symmetry. Finally the curves were studied numerically, as described in the preceding paper (Rosenblueth, 1932), i.e., the constancy of the product $(b - y)(a + x)$ was investigated, where y is the response, x represents the frequency (number of discharges per second), and a and b are constants obtained by the method of least squares. This latter test is the most significant and its results will be given in most instances.

The numerical test for the hyperbolas gave approximations usually closer than 10 per cent, a result quite satisfactory in consideration of the methods employed. The tests for other types of curves gave greater deviations, and, what is more important, the errors were definitely and consistently systematized. This did not occur when they were considered as hyperbolas.

In every case several readings were taken on each frequency. A random order was commonly followed. The results were found to coincide satisfactorily.

The stimulator used was designed and constructed by Mr. E. L. Garceau, electrical engineer, in this Laboratory. The following is Mr. Garceau's description of the apparatus:

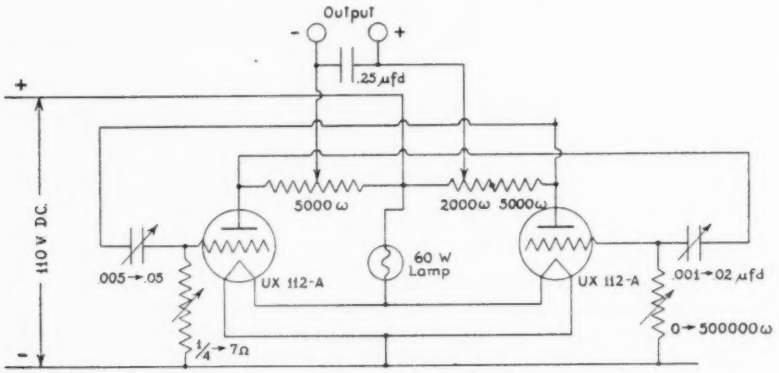
The multivibrator stimulator (see accompanying diagram) is an adaptation of the multivibrator circuit known for some time in electrical communication engineering. It consists essentially of two resistance-capacity coupled amplifier tubes (UX 112A). The plate circuit of each tube feeds into the grid circuit of the other.

In the diagram imagine a minute negative potential from any cause to be impressed upon the grid of one tube. The plate of the tube will then go slightly positive as the space current is decreased, and the grid of the second tube will also go positive. The space current of the second tube will increase, and its plate will therefore go negative, impressing a further negative potential on the first grid. This goes on very rapidly until the first grid is so far negative that the plate current of the first tube is entirely cut off. Now the grid condenser of the first tube discharges its excess charge through the grid leak. During this time there is no plate current in the first tube, but a large one in the second. When the grid condenser has discharged sufficiently to allow the first plate to take current, the grid of the second tube is falling in potential and it blocks in turn. The time of blocking of each tube is nearly proportional to the value of the product of grid leak and coupling capacity of RC. The plate resistances are potentiometers and the stimulating circuit is connected to the moving contacts of these. The circuit is unsymmetrical in that one tube is made to block much longer than the other. Both condensers and grid leaks are variable, and the stimuli are intense pulses of short duration derived from one potentiometer, separated by long intervals during which a small depolarizing voltage flows from the other potentiometer.

The wave form is closely rectangular. The number of stimuli may vary from one in several seconds to 10,000 or more per second. It is difficult to obtain a ratio of

stimulating time to depolarizing time of more than 20 to 1. Either voltage in this apparatus may be run up to about 40 volts. The device may be calibrated very accurately with a cathode ray oscillograph using a linear time axis or with any good photographic oscillograph. It is possible to vary independently the stimulator voltage, stimulating time, depolarizing voltage and time between stimuli. This form of the apparatus works directly from 110 volt direct current line. The plate voltage is derived from a line directly and the filament current is reduced by means of a 60 watt tungsten lamp. It is important to use the correct polarity of the line and to insulate the preparation from ground.

The temperature and the intensity affect the absolute frequency, although the relative scale of the frequencies is not modified. To eliminate any possible error the frequencies were usually recorded and thus determined. The figures of the conventional scale on the dial of the correspond-



Multivibrator Circuit

ing potentiometer varied practically in a linear relation to the intensities of the stimuli, because of the low resistance of this potentiometer. On this scale, 30 was found to be approximately equal to 10 volts. The duration of each stimulus used was from 0.5 to 10σ.

RESULTS. A. STRUCTURES INNERVATED BY THE SYMPATHETIC. *1. Single hair of the tail of the cat.* It was deemed desirable to obtain information on the action of a neuromuscular preparation as simple as possible. A single hair in the tail of the cat was chosen because it is moved by a slender bundle of smooth muscle cells which are parallel and therefore act in one direction only.

Histological evidence was not found in the literature regarding the number of nerve fibers supplying the *arrectores pilorum*. The physiological action,

however, was always, as will be shown below, that of a single neuromuscular unit. Preganglionic fibers were usually stimulated—each fiber being distributed to a larger number of postganglionic elements. In some cases, however, the postganglionic fibers were stimulated and no differences appeared in the observations. If there be a plural postganglionic innervation of the muscle the thresholds of the fibers concerned must be very similar.

One hair was isolated by clipping short all those surrounding it. At rest it lies nearly parallel to the skin and pointing toward the tip of the tail. Stimulation of the lower abdominal sympathetic chains will make the hair describe an arc of a circle whose center is the point of its insertion in the skin.

The movement is at first rapid, then slower, and finally the hair remains at a given position as long as the stimulus is maintained. One minute is usually more than sufficient time to reach this state of equilibrium. Relaxation after the stimulus ceases shows the same, probably exponential, changes in rate, beginning rapidly and becoming continuously slower.

A protractor, held by a clamp so that its diameter was touching the skin, its center coinciding with the base of the hair, and its surface parallel to and close to the plane of movement, permitted the movements of the hair to be read directly and with an accuracy of 0.5°. Records of the movement were regarded as unnecessary since the interesting feature was the maximal excursion of the hair with the different rates of stimulation.

It was assumed that the amount of contraction developed by the muscle is approximately linearly proportional to the angle described by the hair. This assumption rests on the plausible hypothesis of a practically linear resistance offered by the elasticity of the structures opposing the movement. These structures bring the hair back to its resting position when contraction ceases.

If the nerve is stimulated for a certain period (30 to 60 seconds) with a given frequency, and the intensity is gradually increased, there is a narrow threshold zone in which there is gradation of the responses, but the maximal response for that frequency is rapidly reached. Further increases of intensity have no effect. The zone where gradation occurs can be accounted for by summation of subliminal stimuli or by fluctuations in the thresholds and in the refractory periods or, finally, by the influence of the process of depolarization. In no case was there any evidence of steps in the gradation which would imply a plural innervation of the muscle.

Following are the data of a typical observation:

Frequency = 15 shocks per second. The intensities are expressed in the conventional units of the scale of the stimulator (see description above).

INTENSITIES (IN THE ORDER IN WHICH THEY WERE APPLIED)	ANGLES DESCRIBED BY HAIR
10	0°
15	15
12	10
17	16
20	17
13	15
12	12
15	17
10	0
11	5

Single discharges from the stimulator, if long enough to satisfy the "excitation time," will also produce a response. The threshold is then very sharp and the response is maximal from the start. Here again no steps were ever observed.

Following are the data of a typical observation:

Single shocks

INTENSITIES (IN THE ORDER IN WHICH THEY WERE APPLIED)	ANGLES DESCRIBED BY HAIR
5	0°
7	9
10	8
6	0
7	8
9	9
8	9

These reactions to changes in the intensity of the stimulus indicate a simple neuromuscular mechanism, probably a single unit. Also, these results agree with the classical notion of the all-or-none response of the nerve.

The influence of varying frequencies was studied by applying supramaximal stimuli (about twice the threshold) for a time sufficient to produce a state of equilibrium (tetanus). Here a wide-ranged gradation occurs, the height of the contraction increasing with the frequency (summation) until a frequency limit is reached beyond which a sharp initial rise is followed by a rapid decline to a lower level, which may be sustained for some time. The interpretation of this action of too frequent stimuli will be taken up in the discussion; the same phenomenon occurred in all the responses studied.

Within stimulation frequencies that produce a sustained contraction the results of a typical experiment are illustrated in figure 1-A.

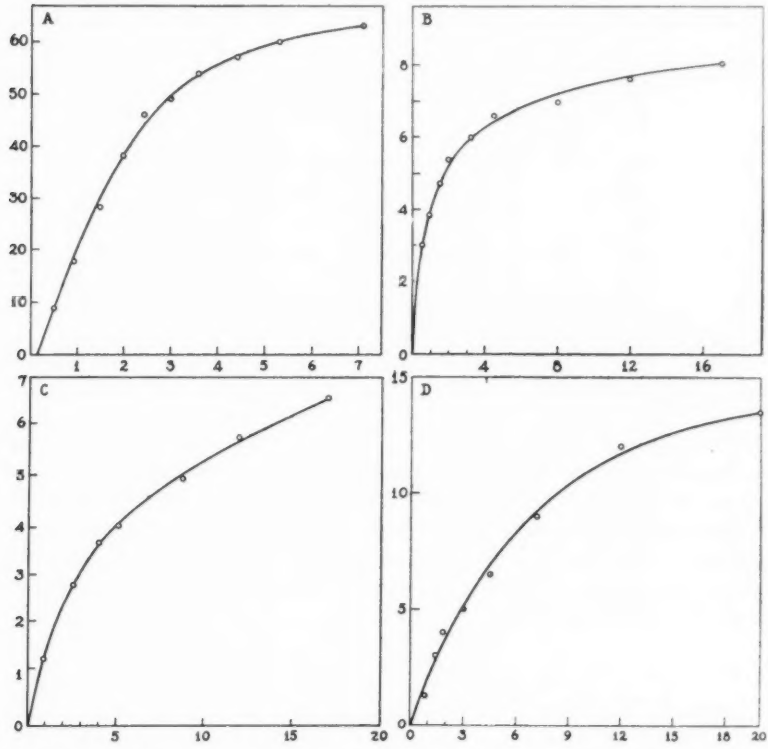


Fig. 1-A. Abscissae: frequencies (shocks per second) of stimulation of the lower abdominal sympathetic chains at L5. Ordinates: angles (in degrees) of movement of a hair in the tail of a cat, averages of several observations. See text for test of this and all other curves.

B. Curve plotted from the results obtained in the experiment illustrated in figure 2. Abscissae: frequencies of stimulation; ordinates: height of contraction in the record 15 seconds after the beginning of stimulation.

C. Curve plotted from the results obtained in the experiment illustrated in figure 3. Abscissae: frequencies of stimulation; ordinates: height of contraction in the record 15 seconds after the beginning of stimulation.

D. Abscissae: frequencies of stimulation of right heart-accelerators. Ordinates: maximal increase of the heart-rate per 15 seconds, averages of several observations.

The method described above to prove that it is a hyperbola yields the following figures:

$a = 1; b = 77.7$

x	y	$(b - y)(a + x)$
0.5	9	103
0.9	20	110
1.5	28	124
2.0	38	119
2.4	46	108
3.0	49	115
3.6	54	109
4.4	57	112
5.3	60	112
7.1	63	119
Average		113

Maximal deviations +11; −10.

2. *Nictitating membrane of the cat.* The distal end of the cut cervical sympathetic was stimulated. Isotonic and isometric contractions were recorded as described by Rosenblueth (1932). An intensity was chosen higher than that necessary to produce a maximal response at a given frequency. This intensity was now applied at varying frequencies. The results are illustrated in figures 1-B and C, 2 and 3. Analysis of the curves gives the following values:

Figure 1-B

$a = 2; b = 8.75$

x	y	$(b - y)(a + x)$
0.5	3.0	14.5
0.9	3.9	14.2
1.4	4.7	13.9
2.0	5.4	13.6
3.2	6.0	14.5
4.6	6.6	14.5
8.0	7.1	16.3
12.0	7.6	16.1
17.0	8.1	14.2
Average		14.6

Maximal deviations +1.7; −1.

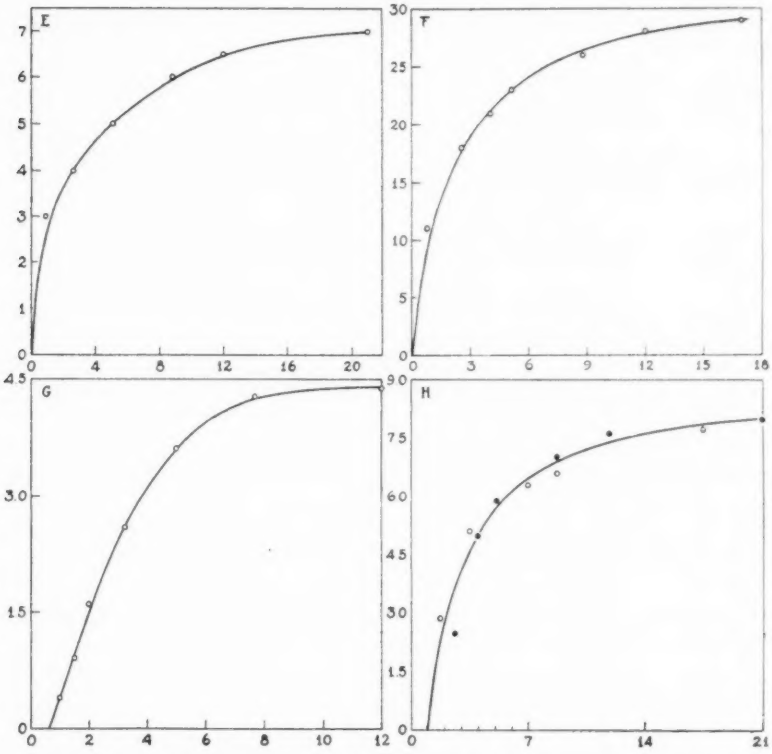


Fig. 1-E. Curve plotted from the results obtained in the experiment illustrated in figure 4. Abscissae: frequencies of stimulation; ordinates: average heights of the contractions in the record.

F. Adrenals removed, right splanchnics cut. Abscissae: frequencies of stimulation of the left splanchnics. Ordinates: average heights of inhibition of the duodenum in the record. See text for explanation of method used.

G. Curve plotted from the results obtained in the experiment illustrated in figure 5. Abscissae: frequencies of stimulation of the adrenal; ordinates: average maximal rises of blood pressure in the record.

H. Abscissae: frequencies of stimulation of the left splanchnics for one minute (dots) and doses of adrenalin injected in one minute (circles), unit of adrenalin 0.000286 mgm. Ordinates: maximal height of contraction of the nictitating membrane in the record. Magnification, 11.

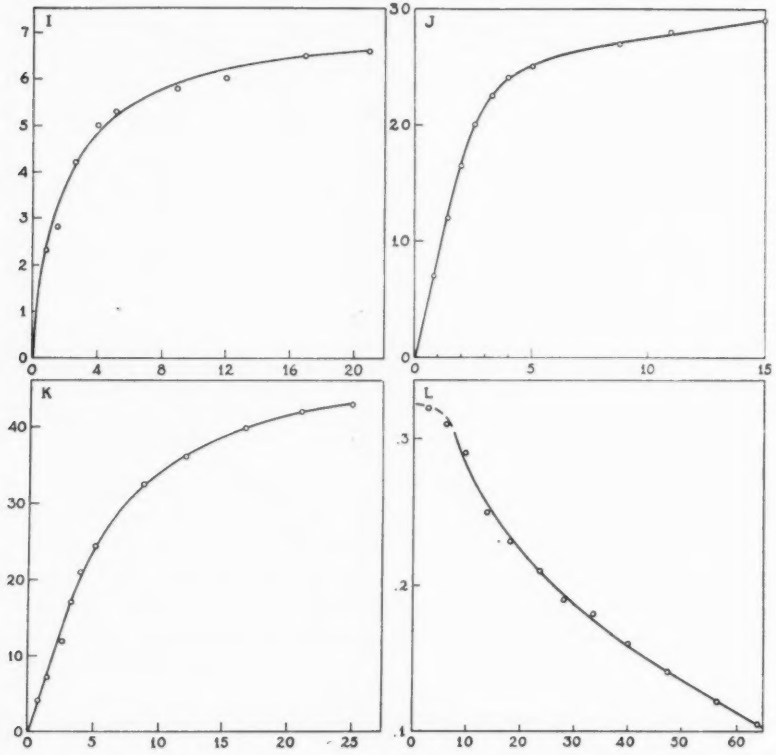


Fig. 1-I. Curve plotted from the results obtained in the experiment illustrated in figure 6. Abscissae: frequencies of stimulation; ordinates: mean height of the contractions in the record.

J. Abscissae: frequencies of stimulation of the right vagus. Ordinates: maximal slowing of the heart-rate per 15 seconds.

K. Curve plotted from the results obtained in the experiment illustrated in figure 7 and from several other observations on the same animal. Abscissae: frequencies of stimulation; ordinates: average total number of drops of saliva secreted.

L. Abscissae: time in millimeters on the record. Ordinates: rate of flow of saliva measured by taking the reciprocal of the intervals between the drops in millimeters.

Figure 1-C

$a = 14.4$; $b = 10.4$

x	y	$(b - y)(a + x)$
0.9	1.3	139
2.6	2.7	131
4.0	3.5	127
5.1	3.8	129
8.8	4.7	132
12.0	5.5	129
17.0	6.3	129
Average		131

Maximal deviations +8; −4.

These are, therefore, again hyperbolas.

Figures 2 and 3 present two features which were consistently found in other experiments. The level of maximal contraction (plateau) is at first horizontal; after a given critical frequency there occurs the usual exponential rise followed by a continuous slow linear ascension, so that the "plateaus" are no longer horizontal. The slope of this slow ascension is steeper as the frequency increases. The second characteristic to be observed in these curves is the longer after-effect as the frequency increases. The explanation of these facts will be taken up in the discussion.

3. *Accelerator of the heart.* The vagi were cut in the neck, after insertion of a tracheal cannula for spontaneous and artificial respiration. The stellate ganglia were approached through the right third intercostal space. The left one was removed. The superior, inferior and external branches of the right one were cut and shielded electrodes were applied to the internal branches. Supramaximal stimuli were applied with varying frequencies.

The results of a typical instance are illustrated in figure 1-D. The usual test for a hyperbola is the following:

$a = 7.4$; $b = 18.6$

x	y	$(b - y)(a + x)$
0.8	1.5	140.2
1.4	3.0	137.3
1.8	4.0	134.3
3.0	5.0	141.4
4.6	6.5	145.2
7.2	9.0	140.2
12.0	12.0	128.0
20.0	13.5	139.7
Average		138.3

Maximal deviations +6.9; −10.3.

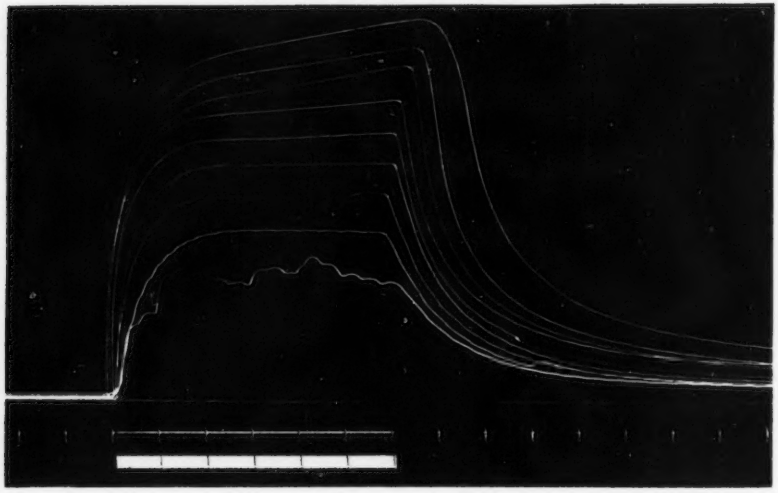


Fig. 2. Isotonic contractions of the nictitating membrane on stimulation of the cervical sympathetic with the following frequencies: 0.5, 0.9, 1.4, 2, 3.2, 4.6, 8, 12 and 17 supramaximal shocks per second. Time recorded in 5 second intervals. Magnification, 12. Tension on muscle, 4 grams.

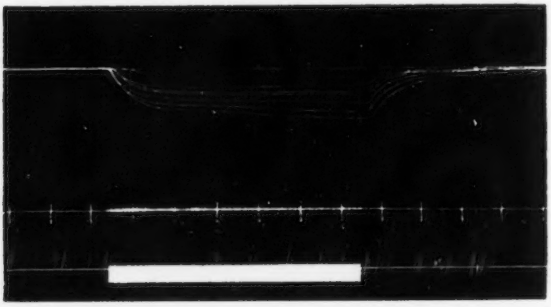


Fig. 3. Isometric contractions of the nictitating membrane on stimulation of the cervical sympathetic with the following frequencies: 0, 0.9, 2.6, 4, 5.1, 8.8, 12 and 17 supramaximal shocks per second. Time recorded in 5 second intervals. Magnification, 9. Tension on muscle, 3 grams. A deviation of 1 cm. in the record is equivalent to a tension of 8.5 grams developed by the muscle.

4. *Contraction of the pregnant uterus of the cat.* The hypogastric nerves, cut or crushed above, were stimulted with supramaximal intensity and varying frequencies. The contractions were recorded by the method described by Rosenblueth (1931).

The results are shown in figures 1-E and 4. The numerical test for a hyperbola is the following:

$a = 4.4; b = 8.1$

x	y	$(b - y)(a + x)$
0.9	3.0	27.1
2.6	4.0	28.7
5.1	5.0	29.4
8.8	6.0	27.7
12.0	6.5	26.3
21.0	7.0	27.9
Average		27.8

Maximal deviations +1.6; −1.5.

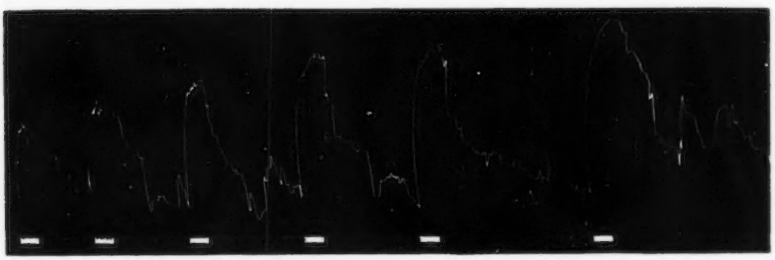


Fig. 4. Isotonic contractions of a cat's pregnant uterus on stimulation of the hypogastric nerves with the following frequencies: 0.9, 2.6, 5.1, 8.8, 12 and 21 supramaximal shocks per second. Time recorded in 1 minute intervals. Magnification, 10. Tension on muscle, 5 grams.

The records are included to show the close similarity of the general shape of the responses with, for example, those of the nictitating membrane, or the stomach on stimulation of the vagus.

5. *Inhibition of the intestine on stimulation of the splanchnics.* The method used to record contraction of the stomach on vagal stimulation (see below) was found inadequate to show inhibition quantitatively. The balloon method was therefore selected. The duodeno-ileum was preferred to the stomach because it presents a higher tonicity during fasting. Anesthetics were avoided because those tried affected the responses. After

dial, for instance, stimulation of the splanchnics or injections of adrena evoke a disappearance of peristalsis or rhythmic contraction, but there no decrease of tone. The cats were therefore pithed (see general method, above). Curare was usually administered; the rhythmic contractions of the intestine decrease slightly in amplitude and frequency, but the tone is not lowered and the responses to nervous stimulation are normal. The adrenals were ligated.

Because of relatively large spontaneous variations of the tone and activity of the organ the quantitation of the responses is not very accurate. A membrane tambour was used as a recording manometer; its calibration with a water manometer revealed a linear direct relation between the deviations of the writing point and the pressures within the range occurring in the experiments. The readings were taken from a mean base line representing tone. Several stimulations were applied with each frequency and averages were obtained.

Figure 1-F represents the curve of a favorable instance. The numerical test is the following.

$a = 3.1$; $b = 33.4$

x	y	$(b - y)(a + x)$
0.8	11	87.4
2.6	18	87.8
4.0	21	88.0
5.1	23	85.3
8.8	26	88.1
12.0	28	81.5
17.0	29	88.4
Average		85.8

Maximal deviations +2.6; −4.3.

6. *Stimulation of the adrenal gland through the splanchnics.* The quantitation of the amounts of adrenin secreted was effected by recording the contractions of the sensitized, denervated nictitating membrane (Rosenblueth and Cannon, 1932) or the rises of blood pressure in the animal sensitized by cocaine. The first test is more exact because in the second important vasoconstrictor influences may increase the response, although in some cases this complication was partly avoided by cutting some of the branches of the corresponding semilunar ganglion. The Elliott (1912) preparation was found unsatisfactory because of the low blood pressure. The following technique makes the changes of blood pressure as sensitive and accurate to adrenin as Elliott's: dial anesthesia is given, the vagi are cut, and cocaine (about 8 mgm. per kilo) is injected intravenously.

The results obtained with either the nictitating membrane or the blood pressure are identical. Figures 5 and 1-G exemplify an experiment with the blood pressure as the indicator. The following is the test of the curve:

$a = 0.55$; $b = 5.1$

x	y	$(b - y)(a + x)$
1.0	0.4	7.3
1.5	0.9	8.4
2.0	1.6	9.0
3.2	2.6	9.4
5.0	3.6	8.3
7.7	4.2	7.4
12.0	4.4	8.8
Average		8.4

Maximal deviations +1; −1.1.

The nictitating membrane permits testing the similarity between the curves obtained by stimulations of the splanchnic with varying frequencies

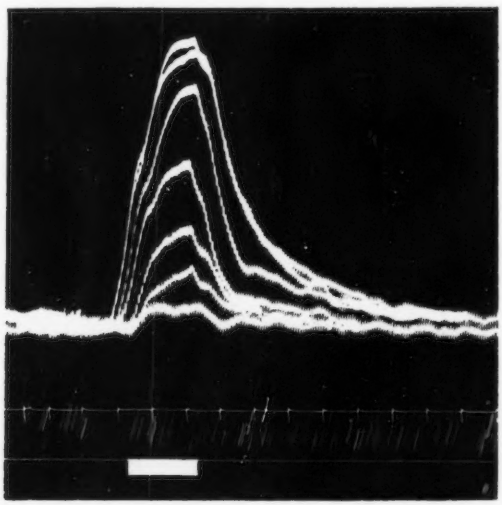


Fig. 5. Cat. Dial, 0.9 cc. per kilo by stomach tube; cocaine, 8 mgm. per kilo intravenously. Vagi cut. Rises of blood pressure on stimulation of the left adrenal medulla through the splanchnics (other branches cut) with the following frequencies: 1, 1.5, 2, 3.2, 5, 7.7 and 12 supramaximal shocks per second. Time recorded in 5 second intervals.

and by injection of varying doses of adrenalin. Figure 1-H illustrates the satisfactory check obtained.

From these data it is possible to calculate the absolute amounts of adrenin secreted by one gland for each stimulus. This amount is 0.0000048 mgm. for this particular experiment and coincides with the average of three observations on different animals.

The maximal amount of adrenin found by Cannon and Rapport (1921) to be secreted reflexly was 0.0037 mgm. per kilo per minute. This figure implies about 10 nerve impulses per fiber per second for a cat of average weight (3.5 kilos). This figure agrees with recent research on the physiological rate of discharge of sympathetic nerves (cf., for instance, Adrian, Bronk and Phillips, 1932).

In the following numerical test of curve 1-H all the points are taken, both those corresponding to stimulation of the splanchnics and those obtained by the injections of adrenin. Now x represents either frequency per second or amounts of adrenin in conventional units (1 = 0.000286 mgm.). The asterisks mark the frequencies.

$a = 1.4; b = 9$

x	y	$(b - y)(a + x)$
1.75	2.8	19.5
*2.6	2.5	26.0
3.5	5.3	18.1
*4.0	5.0	21.6
*5.1	5.8	20.8
7.0	6.3	22.7
8.7	6.6	24.2
*8.8	7.0	20.4
*12.0	7.6	18.8
17.5	7.7	24.6
*21.0	8.0	22.4
Average		21.7

Maximal deviations +4.3; −3.6.

B. Structures innervated by the parasympathetic. 1. *Contraction of the stomach.* Cats (fasting 24 hours) were used, usually under dial anesthesia. The splanchnics were cut. The vagi, crushed or cut above, were stimulated immediately below their entrance into the abdomen. The movements were recorded by the method described by Rosenblueth (1931). Notwithstanding the complicated arrangements of gastric muscle fibers, the movements of the writing lever can be satisfactorily interpreted since the main component of the rise is the contraction of the circular

muscle rings at the level where the thread to the lever is attached. The rise of the lever is therefore lineally proportional to the shortening of the radius of this circle, or to the circumference, or, finally, to the actual shortening of the circular muscular fibers.

The results of a typical case are illustrated in figures 6 and 1-I. Again there is a striking similarity between these records and those obtained with the uterus or with the much simpler nictitating membrane.

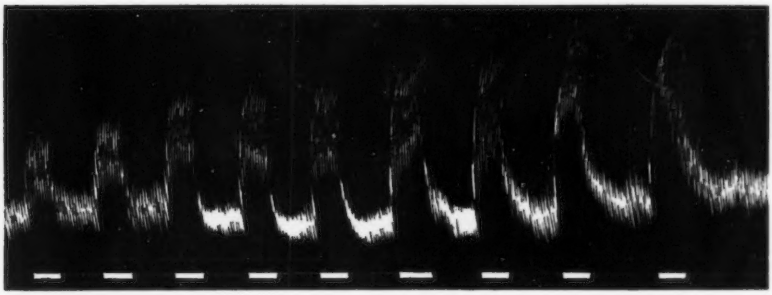


Fig. 6. Isotonic contractions of the circular muscle fibers of the stomach on stimulation of the vagi in the abdomen with the following frequencies: 0.9, 1.5, 2.6, 4, 5.1, 8.8, 12, 17 and 21 supramaximal shocks per second. Time recorded in 30 second intervals. Magnification, approximately 7. Tension on muscle, approximately 6 grams.

The test of curve 1-I for a hyperbola yields:

$a = 1.7; b = 7.2$

x	y	$(b - y)(a + x)$
0.9	2.3	12.7
1.5	2.8	14.0
2.6	4.2	12.9
4.0	5.0	12.5
5.1	5.3	12.9
8.8	5.8	14.7
12.0	6.0	16.4
17.0	6.5	13.1
21.0	6.6	13.6
Average		13.6

Maximal deviations +2.8; −1.1.

2. *The action of the vagus on the heart.* Both stellates were removed in a cat under dial. The vagi were cut in the neck and shielded electrodes

were placed distally on each nerve. The rate of the heart was taken by means of a carotid cannula connected to a mercury manometer. Supramaximal stimuli at varying frequencies produced comparable results with either vagus, though quantitatively greater for the right one.

Figure 1-J shows the results obtained. The test of the curve is the following:

$a = 0.6$; $b = 31.2$

x	y	$(b - y)(a + x)$
0.8	7.0	33.9
1.4	12.0	38.4
2.0	16.5	38.2
2.6	20.0	35.8
3.3	22.5	33.9
4.0	24.0	33.1
5.1	25.0	35.3
8.8	27.0	39.5
11.0	28.0	37.1
15.0	29.0	34.3
Average		35.9

Maximal deviations +3.6; −2.8.

3. *The submaxillary gland.* Dogs were usually employed because the larger amounts of saliva obtained decreased the experimental error. Cats, however, presented similar results.

Figures 7 and 1-K show the results of a typical observation. The test of the curve is the following:

$a = 4.37$; $b = 52.5$

x	y	$(b - y)(a + x)$
0.8	4.0	250
1.5	7.0	267
2.6	12.0	280
3.3	17.0	273
4.0	21.0	265
5.1	24.5	263
8.8	32.5	264
12.0	36.0	270
16.8	40.0	265
21.0	42.0	267
25.0	43.0	279
Average		267

Maximal deviations +13; −17.

The lingual nerve was cut above and below the emergence of the chorda tympani and the latter was stimulated with supramaximal intensities and varying frequencies. Wharton's duct was cannulated and connected with a long tube filled with water (colored by methylene blue) in order to obtain drops of the same size. The drops were recorded by making them fall on a lever attached to a receiving tambour.

The similarity between these results and those obtained in muscle (say, the nictitating membrane) is striking. With the increasing frequency of stimuli the response is earlier, the after-effect longer. The shape of the after-effect in relation to time is shown in figure 1-L; it is an exponential of the formula $y = ke^{-k't}$, identical with the formula of relaxation of smooth muscle (Rosenblueth, 1932).

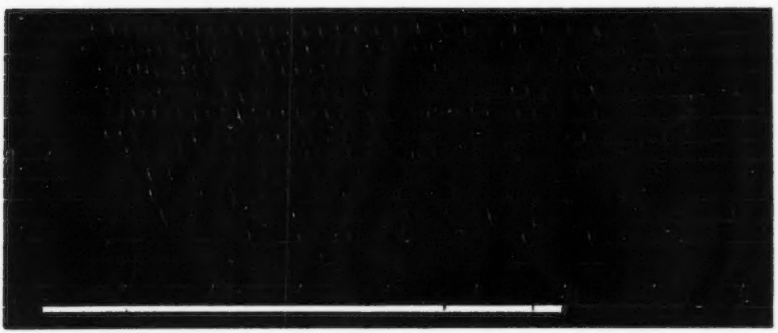


Fig. 7. Dog. Drops of saliva obtained on stimulation of chorda tympani with the following frequencies, from below upward: 0.8, 1.5, 2.6, 3.3, 4, 5.1, 8.8, 12, 16.8, 21 and 25 supramaximal shocks per second. Time recorded in 5 second intervals. At the higher frequencies (from 8.8 on) the last drops do not appear on this record. Twelve drops = 1 cc.

Discussion. In the following discussion it will be shown, first, that the commonly accepted interpretations are not adequate to explain the data presented. The hypothesis of a chemical mediation will then be examined and some of its consequences will be stressed. Some particular instances will be dealt with separately.

The experiments performed on muscles have been dealt with by previous workers under the heading of summation of the responses, a steady state of contraction (tetanus) being interpreted as a fusion of single twitches. It is shown in this paper that the relations between the frequency of the nervous stimuli and the magnitude of the responses obey a general hyperbolic law. This law applies to as widely different types of responses as contraction, inhibition, secretion and the rate of the heart. The only exception found to the law, adrenal secretion, will be discussed later.

If a simple hypothesis, applicable in all cases, will explain all the phenomena observed, it should be preferred to any explanation which can cover only one of the cases. This consideration alone is sufficient to lessen the value of such theories as make a peculiar characteristic of the structure considered (say, the muscle) the basis on which they rest. They will, however, be included in this discussion.

Before discussing summation of responses it is, however, necessary to consider whether the phenomenon of summation of nervous stimuli is not concerned in these cases. The affirmation that the structures innervated by the autonomic nervous system respond only to repeated nervous stimuli occurs frequently in the literature (see, for instance, Lapicque and Meyerson, 1921; Lapicque, 1925; Fredericq, 1928; Bremer and Homès, 1931). We agree with Bishop and Heinbecker (1932) that this statement is incorrect. As shown by Stewart (1900), the cat's bladder contracts sharply in response to a single induction shock. In our experiments the nictitating membrane and the pilomotors reacted definitely to single shocks. So did the heart to vagal inhibition. In the case of the submaxillary gland stimuli separated by as much as three seconds will evoke a definite response if repeated sufficiently. The repetition is necessary to make the secretion apparent because of the minute amounts which would be produced by each stimulus, for the intervals are probably long enough to exclude summation. In fact, a response was invariably obtained in every structure examined with frequencies as low as one shock every one or two seconds. This makes it improbable that summation of stimuli played a rôle in any case. Finally, even if it did, whatever the theory adopted for the summation of nervous stimuli, the only consequence in relation to the responses to varying frequencies would be a change of scale. This improbable possibility will therefore be disregarded.

Fulton (1925) explained summation in muscle as due to the viscosity of the tissue and its changes during contraction (see Gasser and Hill, 1924). This theory is inadequate for either skeletal or smooth muscle because, if it were correct, the maximal tension possible would always be attained, independently of the frequency, after a lower frequency limit, necessary for summation to occur at all, has been reached. The only difference between slower and faster rates of stimulation would be that the former would produce a maximal tension after a longer period of application than the latter. This consequence of the theory is in disagreement with all experimental evidence.

Mines (1913) proposed the hypothesis that summation in skeletal muscle is due to an increased C_H (quantal releases of lactic acid at each stimulus). The contraction would then be proportional to the C_H of the muscle. This is a chemical mediation theory. It is inacceptable because of the large amounts of lactic acid which would be necessary to change the pH. Be-

sides, it leads to a linear or parabolic relation between the frequencies and the responses (cf. any work on physical chemistry), which is not confirmed by experience.

Hill (1913) showed that, although heat-production and tension increase with frequency, the heat-production per unit of tension developed is constant. He therefore concluded that the rise of tension is due to the presence of chemical substances, liberated in conjunction with heat by the processes called forth by excitation. The amount of tension developed is proportional to the amount of these substances present. This theory was again taken up by Hartree and Hill (1921) and slightly enlarged and modified, as follows. Let contraction be due to the appearance of substance B (lactic acid) at certain surfaces of the muscle. In relaxation B is destroyed or removed from its seat of action. B is produced from A, of which there is a large supply in the muscle, according to the reaction $A \rightarrow B$, under the influence of a catalyst. Equilibrium exists in a space impermeable to B. A shock produces a certain momentary permeability. If now the shock is repeated the amount of B which passes out is smaller. Equilibrium will occur independently of the frequency when all B has escaped, and is conditioned only by the speed of the reaction $A \rightarrow B$.

The hypothesis is not very explicit as regards our present problem. If the last statement be taken textually the assumption is inadmissible, for equilibrium (i.e., sustained constant tension during stimulation) *is* dependent on frequency of stimulation (see, for references on skeletal muscle, Davis and Davis, 1932). The amounts of B diffusing at each shock will only be smaller with successive stimuli if the frequency is greater than the velocity of the reaction $A \rightarrow B$. If the only factor were this velocity, regardless of frequency, equilibrium would always be attained at the same amount of tension (i.e., the maximal tension would always be reached) and this is not the case.

The hypotheses examined do not, therefore, agree with experimental evidence. Before analyzing the theory of a chemical mediation it now becomes necessary to examine the part the nervous impulses elicited by the electrical stimuli may play in explaining the curves obtained. This can be only minimal and negligible. Gerard, Hill and Zottermann (1927) found a decrease of the electrical response and the heat produced for a single nervous impulse (and therefore also probably the "intensity" of the impulse) as the frequency increased. At the rates used in these experiments however (i.e., successive impulses occurring well beyond the refractory period of the nerve), this decrease can be neglected. The effective inflexion toward the asymptote in Gerard, Hill and Zottermann's curve occurs when the limits of the refractory period are reached.

The theory of a chemical mediation adopted here can be expressed as follows. Each quantal (all-or-none) nervous impulse liberates a quantal

(constant) amount of a chemical mediator, M. M combines with some hypothetical substance H in the effector according to the reaction $M + H \rightleftarrows MH$. The response is proportional (not all-or-none) to the amount of MH formed. Free M is destroyed locally; this will produce the direction $\leftarrow$ of the reaction when no more M is supplied and relaxation will ensue. The amount of M which can be destroyed locally at a given time is limited; hence if M should occur at a higher concentration than this amount it will diffuse into the blood where it may be destroyed or whence it may diffuse into other structures and produce its characteristic effects.

The hypothesis leads to the following formula for the relations between the height of the response R and the concentration of (M): $R = \frac{(M)}{k - k'(M)}$. But $(M) = qF$ (frequency). Hence, $R = \frac{F}{k - k'F}$. This is the formula for a rectangular hyperbola whose asymptotes are parallel to the axes. It has been shown in the data presented in the foregoing pages how fully experimental evidence confirms these relations.

The shape of the responses, the increasing after-effects with higher frequencies, the constant linear rise of the responses with frequencies higher than a certain critical level, are all accounted for satisfactorily. The direct evidence in favor of the adequacy of the explanation is the demonstration of autonomomimetic effects in denervated organs after autonomic stimulation elsewhere: vagal and sympathetic substances (Loewi, 1921), sympathin (Cannon and Bacq, 1931), chorda-hormone (Babkin, Stavraky and Alley, 1932). The after-effects are longer in stimulation of the sympathetic (see figs. 2 and 3) or of the chorda tympani (see fig. 7) than in stimulation of the vagal territory (heart, stomach, see fig. 6 and pp. 26–28). This would indicate a more ready destruction of the mediator in the latter system. It is interesting to correlate this fact with Freeman, Phillips and Cannon's (1931) fruitless attempt to show a vagus hormone in conditions similar to those in which sympathin is readily demonstrated.

The effect (see p. 16) of excessive frequencies of stimulation (i.e., a maximal response followed shortly by a slow and continuous fall) coincides with that found by Orías (1932) on the nictitating membrane and by Davis and Davis (1932) on skeletal muscle. Its interpretation need not be discussed here. It is evident that after a certain frequency the refractory period of the muscle, or of the ganglion if preganglionic fibers are stimulated, probably increased by previous stimulation, will play a definite part in producing the effect.

Bishop and Heinbecker (1932) state that they do not believe that accurately interpretable results can be obtained by stimuli on the cervical sympathetic more frequent than 10 per second. Our experiments (see

figs. 1-B and C, 2 and 3) would increase this limiting frequency to 20 per second for the preganglionic fibers in the cervical sympathetic. These figures agree with the values found by Bishop and Heinbecker (1930) for the refractory periods of the ganglion synapse and the postganglionic fibers (20 to 30 and 4.5 σ, respectively). We agree with these authors (1932) that the results of Querido (1924) and Veach and Pereira (1925) with much higher frequencies are inacceptable.

The approximate frequencies found to produce a maximal response in our experiments were, for the preganglionic fibers, the following:

STRUCTURE	FREQUENCY PER SECOND
Sympathetic	
Pilomotors	15
Nictitating membrane	20
Pregnant uterus (postganglionic)	20
Intestine	20
Adrenal medulla	25
Heart (probably postganglionic)	25
Parasympathetic	
Heart	30
Submaxillary gland	36
Stomach	25

The maximal frequency effective on the preganglionic fibers is the only one that is interesting since, as Bishop and Heinbecker (1932) show, the longer refractory period of the ganglion will be the determinant of this maximal frequency, and there is no after-discharge from the ganglion.

The chemical theory adopted makes the process of relaxation an active one: it is rather a progressive "decontraction" conditioned by the gradual disappearance of the mediator than an actual sudden relaxation, the shape of which would then be conditioned by the viscosity of the muscle. This view is corroborated by the increasing after-effects with higher frequencies (see figs. 2, 3, 4 and 6).

This concept of the disappearance of the response is undoubtedly true in the case of the submaxillary gland (see fig. 7). The rate of the disappearance here as elsewhere is an exponential (see fig. 1-L). The interpretation of the responses of the submaxillary gland must, however, be dealt with more extensively. The hyperbola presented in figure 1-K was obtained by plotting the total amounts of saliva produced against the frequency. This total amount is not comparable with the maximal height

of the contraction of a muscle, but rather with the area of the record of a contraction. These areas can be readily obtained with a planimeter. Their relations to the frequency or to the amounts of chemical mediator or of adrenin injected can be obtained by integrating the formula for contraction (see Rosenblueth, 1932)

$$y = \mu \left[\frac{k(M)X}{k' + k(M)} \left(1 - e^{-(k' + k[M])t}\right) \right]$$

between the limits 0 and t_1 (end of contraction), and the formula for relaxation

$$y = (MH)_o \, e^{-k't} = \mu \frac{k(M)X}{k' + k(M)} e^{-k't}$$

between the limits t_1 and ∞, and adding. Calling A the area we then have

$$A = \int_0^{t_1} \mu \left[\frac{k(M)X}{k' + k(M)} \left(1 - e^{-(k' + k[M])t}\right) \right] dt + \int_{t_1}^{\infty} \mu \frac{k(M)X}{k' + k(M)} e^{-k't} dt =$$

$$= \frac{\mu k(M)X}{k' + k(M)} \left[t_1 + \frac{e^{-k't_1}}{k'} + \frac{1}{k' + k(M)} \left(1 + e^{-(k' + k[M])t_1}\right) \right]$$

If we now consider that t_1 varies only slightly and in the same direction as M, and that M is exceedingly small compared to the other values concerned (see, for instance, the figures obtained for the output of adrenin by the adrenal gland per stimulus (p. 26); M would probably be still smaller), we can consider the expression in the parenthesis to be practically constant. The formula then becomes

$$A = \frac{(M)}{k' - k(M)}$$

that is, it again yields a hyperbola.

This is what curve 1-K illustrates for the salivary gland. This procedure was likewise applied to several curves obtained from contractions of the nictitating membrane to varying frequencies of stimulation or to varying doses of adrenin. The areas of these contractions, which represent the work done, produced similar curves.

If, on the other hand, tension in muscle be legitimately compared to flow in the salivary gland, it is sufficient to plot the number of drops for a given time during stimulation, or the intervals between these drops, against the frequencies, to make the situation comparable to that of the muscle. If this is done hyperbolas are again obtained. The total response

was used, and not these latter methods, because of simplicity and greater accuracy in quantitating the response, since the flow is not constant. The fact that the first drops occur at a faster rate than the subsequent ones can possibly be explained as due to squeezing of saliva from the ducts by vasodilatation. The flow later (after about 30 seconds) becomes steady. It requires very long stimulation (over 10 minutes) for the exponential decrease due to fatigue to appear.

The all-or-none law is rejected for smooth muscle by the interpretation adopted. The same conclusion was reached in the case of the action of adrenin (Rosenblueth, 1932). In this relation we would mention that Bishop and Heinbecker (1932) repeatedly state that smooth muscle does not follow the all-or-none principle, without, however, giving any evidence, arguments, or references.

The secretion of the adrenal medulla offers an interesting exception to the hyperbolic law. It was shown (Rosenblueth, 1932) that varying concentrations of adrenin in the blood bear a hyperbolic relation to the responses of the indicator. Since varying frequencies of stimulation of the splanchnics again elicit reactions of the indicator which yield a hyperbola (see figs. 1-G and H, and 5 and pp. 25, 26) it follows that the amounts of adrenin secreted are linearly proportional to the frequency. This atypical behavior is possibly correlated with the atypical innervation of the gland, by preganglionic fibers only (Elliott, 1913), and with the embryological identity of the adrenal medulla and the postganglionic neurones. In accord with this suggestion the production of the mediator might depend on the postganglionic neurone.

Bishop and Heinbecker (1932) state that no difference can be detected by observation of the end response when, beginning with a submaximal intensity, either the frequency or the intensity of stimulation of the cervical sympathetic is increased. From this they conclude that temporal and spatial summation amount to the same values. We must disagree with this conclusion. If the optimal frequency is chosen and the intensity increased the maximal response will be obtained, but it will be due to spatial *plus* temporal summation. If, however, the frequency is low, say 2 per second in any of our experiments, spatial summation alone will never produce the maximal response.

Spatial and temporal summation can only be legitimately compared when acting independently, but this comparison cannot be done experimentally. Exclusive temporal summation is simple to analyze; the pilomotor responses are a good example of it since the muscles are probably innervated by a single fiber (see p. 16). But the effects of pure spatial summation will vary with the number of fibers innervating the structure considered. It can, however, be stated that the maximal effects of pure spatial summation in the autonomic nervous system can be considerably increased if temporal

summation comes also into play. The maximal height of contraction of the nictitating membrane obtained by stimulation at the optimal frequency is usually 10 to 15 times greater than that of a "twitch" in response to a single supramaximal stimulus. In the case of the submaxillary gland this ratio is obtained by comparing the flow of saliva during stimulation at the optimal frequency and after a single supramaximal shock (see p. 34). In one experiment the flow was 0.066 drop per second when stimulated at the frequency of 1 per second and 1.25 drop per second when the frequency of stimulation rose to 30. This would give a ratio of 19, which could be still increased by slowing further the lower frequency.

Our conclusion is then that spatial summation in the autonomic nervous system is of scant value if compared with the effects of temporal summation.

SUMMARY AND CONCLUSIONS

The relations between the responses and the frequency of supramaximal stimulation of autonomic nerves were studied in the following cases: a single hair in the tail of the cat (see fig. 1-A and pp. 14–18), the nictitating membrane (isometric and isotonic contractions, see figs. 1-B and C, 2 and 3 and pp. 18–21), sympathetic acceleration of the heart (see fig. 1-D and p. 21), isotonic contractions of the cat's pregnant uterus (see figs. 1-E and 4 and p. 28), inhibition of the duodenum (see fig. 1-F and p. 24), the adrenal medulla (see figs. 1-G and H and pp. 24–26); vagal stimulation of the stomach (see figs. 1-I and 6 and pp. 26, 27), vagal inhibition of the heart (see fig. 1-J and p. 28), and the submaxillary gland (see figs. 1-K and 7).

The shapes of the records of the muscular responses are exponentials, with the formulae $y = k(1 - e^{-k't})$ for the contraction and $y = ke^{-k't}$ for the relaxation (see figs. 2, 3, 4 and 6). The second formula is also applicable to the rate of disappearance of the response of the submaxillary gland after stimulation (see fig. 1-L).

With the exception of medulliadrenal secretion, which is in a linear relation with the frequency (see p. 35), all the systems investigated obey the following hyperbolic law $R = \frac{F}{k' - kF}$, where R is the maximal height of the response, F the frequency of stimulation, and k' and k, constants (see figs. 1-A to K).

Summation of nervous stimuli probably does not occur in these experiments (see p. 30).

Several theories of summation of responses, proposed by various authors, are examined and found inadequate to explain the experimental data presented in this paper (see pp. 30, 31).

The characteristics of the nervous impulses elicited by the stimuli probably do not account for the phenomena observed (see p. 31).

The following chemical hypothesis is proposed. Each quantal nervous impulse liberates a quantal amount of a chemical mediator, M. M combines with some substance H in the effector according to the reaction $M + H \rightleftarrows MH$. The response is proportional to the concentration of MH, not all-or-none. Free M is destroyed locally, hence relaxation. This destruction occurs at a limited rate, hence possible diffusion to other structures when the concentration exceeds this limit.

This hypothesis accounts for all the data observed: the exponential shape of the responses, the hyperbolic ratio between the maximal heights and the frequencies, the constant linear rise of the responses with frequencies higher than a certain critical level (see figs. 2 and 3 and p. 21), and the increasing after-effects with increasing frequencies (see figs. 2, 3, 4 and 6 and p. 21). The autonomomimetic effects in denervated organs after autonomic stimulation elsewhere in the organism are direct evidence which supports the hypothesis (see p. 32 for references).

The average optimal frequencies found in these experiments are given on page 33.

It is shown that relaxation of smooth muscle is probably an active process, a "decontraction," comparable to the disappearance of the salivary flow after stimulation of the chorda tympani (see fig. 1-L and p. 33).

The area of a contraction is obtained by integration of the corresponding formulae. This area represents the work performed, and is comparable to the total amount of saliva secreted during stimulation. It is shown that there exists again a hyperbolic relation between the contraction areas or the total amounts of saliva and the stimulation frequencies (see fig. 1-K and pp. 33, 34).

Spatial and temporal summation in the responses of organs supplied by the autonomic nervous system is discussed in pages 35, 36. It is concluded that the effects of spatial summation are small when compared with those obtained by temporal summation.

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THE EFFECT OF PROGESTIN-CONTAINING EXTRACTS OF CORPORA LUTEA ON UTERINE MOTILITY IN THE UNANESTHETIZED RABBIT WITH OBSERVATIONS ON PSEUDOPREGNANCY[1]

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In a previous series of experiments one of us has shown, by the uterine fistula in the unanesthetized rabbit, that the uterus usually undergoes strong, rhythmical contractions when the animal is in heat[3] and that within a few hours after copulation this motility gradually decreases in intensity so that by the time ovulation has occurred, or even before, the uterus is quiescent (Reynolds and Friedman, 1930a, 1930b). This period of inactivity persists throughout pseudopregnancy, but as the does again come into heat, rhythmical motility returns. After castration the motility also disappears within 2 to 3 days, but if Theelin is injected intravenously into the recently castrated animal, as little as 2 to 5 r.u. per kilo causes a return of motility within 24 hours (Reynolds, 1931a). This series of experiments shows that rhythmical motility is present when does are in heat, and that in castrated does the injection of Theelin restores this motility. The natural conclusion is that the motility is due to the oestrin present in the animal and that it is one of the physiological manifestations of oestrus. It was later found that, whereas the quiescent uterus of the castrate may be

[1] The previous papers of the series on the hormone, progestin, are listed in the bibliography as follows: I, Corner, 1928; II, Corner and W. M. Allen, 1929; III, Allen, W. M. and Corner, 1929; IV, Goldstein and Tatelbaum, 1929; V, W. M. Allen, 1930a; VI, W. M. Allen, 1930b; VII, Allen, W. M. and Corner, 1930; VIII, Allen, W. M., 1932. Previous work on uterine motility in the unanesthetized rabbit has been summarized in a short article by one of us (S.R.M.R.) which appeared in Endocrinology, xvi, 193, 1932.

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I am indebted to Doctor George L. Streeter, Director of the Department of Embryology, Carnegie Institution of Washington, for extending to me the privilege and full use of the excellent facilities of that laboratory throughout this year.

[3] The term "heat" or "oestrus" as used in this paper refers to a condition in the female rabbit when a sexually mature doe is, in addition to being willing to mate, able to ovulate in response to the normal stimulus of coitus (see Reynolds, 1931a, p. 715).

restored to oestrous motility by the injection of 2 to 5 r.u. of Theelin per kilo, the quiescent uterus of pseudopregnancy is completely refractory to Theelin, even to doses of 1090 r.u. per kilo. This of course indicates that during the pseudopregnancy some substance or condition is present which brings about this tremendous change in susceptibility to Theelin, and that perhaps the inhibiting substance or mechanism originates in the corpus luteum. This is borne out by the fact that removal of the ovaries (hence, corpora lutea) at this time renders the uterus susceptible to small amounts (5 r.u. per kilo) of Theelin (Reynolds, 1931b). Regardless of the simultaneous occurrence of this inhibition of motility and the presence of corpora lutea, however, there have been no experiments which directly show that the inhibitory substance is produced by the corpus luteum.

In another series of papers the other of us, in collaboration with Dr. G. W. Corner, has described the preparation of active corpus luteum extracts which, when injected into rabbits castrated 18 to 24 hours after mating, regularly produce progestational proliferation of the endometrium and bring about normal implantation of the blastocysts and development of the fetuses even to full term. These extracts contain, therefore, a hormone which produces in the castrated rabbit cytological and physiological conditions identical with those found during pregnancy or pseudopregnancy, and because of this fact, the hormone was named progestin, i.e., a substance which favors gestation.

The problem in this paper is to ascertain whether or not accurately assayed progestin-containing extracts will inhibit normal oestrous motility following injections of Theelin and, if such inhibition is observed, to obtain some evidence if possible on the nature of the substance responsible for the inhibition.

The corpus luteum extracts used in this work were made by the method previously described. The corpora lutea of swine are extracted with boiling neutral alcohol. The alcohol is distilled off and the residue extracted with ether. The phospholipids are removed from the ether solution by precipitation with acetone and the acetone-ether soluble oil thus obtained is freed from cholesterol and a large amount of neutral fat by freezing from 70 per cent methyl alcohol. The methyl alcohol soluble substances, after removal of the methyl alcohol, are extracted with ethyl ether and the ether washed with sodium bicarbonate solution. One rabbit unit of progestin prepared by this method usually is equal to about 6 to 8 mgm. of solids. The specific corpus luteum extracts used were of two distinct degrees of purity. Extracts 87 and 92 were so-called crude oil, fraction i; 1 rabbit unit = 400 mgm.) and extract 90 was more highly purified, fraction o; 1 rabbit unit = 8 mgm.) (W. M. Allen, 1932b). Extracts 87 and 92 produced mucification of the vaginas of castrate mice when a suitable dose was injected for 8 days, and cornifica-

tion when larger doses were given. Extract 90 was not tested for its oestrin content, but other extracts made in exactly the same manner have produced either mucification or cornification depending on the dose. These extracts, therefore, contained at least two well-known hormones, progestin and oestrin (Meyer and Allen, 1932).

The Theelin (50 r.u. per cc.) used in these experiments was supplied by Parke, Davis & Co., and has been shown to be a chemically pure substance having the empirical formula $C_{18}H_{22}O_2$. This crystalline hormone was originally isolated by Veler, Thayer and Doisy (1930) and independently by Butenandt (1929) and Laqueur (1930). Another, more definitive name, ketohydroxyoestrin, has recently been proposed for this hormone by Marrian (1932).

Effect of progestin on spontaneous oestrous motility (table 1, fig. 1). For the study of progestin-containing extracts on normal motility 10 oestrous (post partum) rabbits were used whose ovaries were intact and whose uterine motility records were of the normal oestrous type at the time injections were begun. They were then injected subcutaneously once daily for 2 to 3 days and records of the uterine motility made 24 hours after each injection, and daily after injections were stopped. From perusal of table 1 it is seen that the marked pre-injection motility was much diminished 24 hours after the first injection in most cases, although some animals showed arrhythmical motility, and that upon a second and a third injection the motility was still further reduced and in some cases the uterus was quiescent. Normal motility reappeared from two to five days after stopping the injections of progestin. One aspect of the effect of progestin on spontaneous motility is not revealed in table 1. Records of motility were made in most of the above instances at the intervals of three, eight and twelve hours after the subcutaneous injection of progestin. It was found in all cases but one that nearly complete quiescence was attained in the interval of eight to twelve hours after the injection. A slight increase occurred by twenty-four hours (the time first noted in the table) after the injection, and so table 1 indicates motility which is really a partial recovery from the first injection. This motility was in turn overcome by the subsequent injections of progestin. This was especially exemplified by rabbits 22 and 28, which from the table appear to have barely responded to the first injection, yet the uteri of these animals showed only feeble motility some five to ten hours after the injection of progestin. Owing to this wide variation in the time of appearance of diminished motility and in the degree of response we do not attach much importance to the absolute time of first appearance of the inhibitory effect of progestin, for admittedly the mode of administration is artificial and there is as yet no reason to suppose that the single injection does more than roughly approximate the normal rate of formation of progestin by the corpus luteum. The significance of the

TABLE 1

Effect of progestin on spontaneous motility of the uterus

Types of activity may be designated in the following manner:

0 = Quiescent condition of uterus in which no activity can be recorded with our system.

+ = Feeble activity is that which with our system of recording gives records of contractions of ½ inch or slightly less.

++ = Moderate activity is that which varies between ½ inch to 2 inches, but which appears to average 1 inch to 1½ inches.

+++ = Marked activity is that which varies between 2 inches to 4 inches.

++++ = Marked activity, over 4 inches.

In all these classes of activity one may find rhythmical (regular) activity, or arhythmical (irregular) activity. Frequently with feeble activity one sees a sort of undulating movement, constant in rate in any one animal for a short period of time, but varying in rapidity from animal to animal.

RABBIT NUMBER	AMOUNT OF PROGESTIN DAILY IN RABBIT UNITS	NUMBER OF DAYS INJECTED	MOTILITY BEFORE INJECTION	DAILY MOTILITY 24 HOURS AFTER EACH INJECTION OF PROGESTIN			DAILY MOTILITY 48 HOURS AND LATER FOLLOWING LAST INJECTION OF PROGESTIN			
				1	2	3	1	2	3	4
22	0.2	2	++++ 60″–80″	+++ irregular	+ irregular	0–+ irregular	0			
23	0.2	3	++++ 40″–45″	0–+ irregular	++ irregular	+ irregular	0–+ irregular	+++ 60″–70″	++++ 50″–60″	
24	0.2	3	++++ 35″–40″	+–++ irregular	+–++ irregular	chewed fistula (no more records)				
25	0.2	2	++++ 50″	+–++ 35″ and irirregular	+ irregular				peritonitis; discarded records of return of motility	

26	0.2	2	++ irregular	0–+ irregular	0–+ irregular		0–+ irregular	++ 60″	+++ 60″	+++ 60″
27	0.2	2	+++ 50″–55″	+ 50″	0		+ irregular	+++ irregular		
28	0.2	2	++++ 60″	+++ irregular	+–++ irregular		++++ 100″–120″	++++ 30″–40″		
36	1.0	3	+++ irregular	+ irregular	+ irregular	+ irregular	0	+++ 60″–80″	+++–++++ 60″–80″	
37	1.0	3	++ 60″	0	+ irregular	0	+ irregular	+ irregular	++–+++ irregular	++++ 60″
38	1.0	3	+++–++++ 60″	++–+++ irregular	0–+ irregular	0–+ irregular	+ irregular	+ irregular	+–++ irregular	+++ 35″–45″

Nos. 22–28 treated with purified extract 90 (1 rabbit unit = 8 mgm).
Nos. 36–38 treated with crude extract 92-A (1 rabbit unit = 400 mgm.).

occurrence of the inhibitory effect before the time when it is known that proliferation occurs will be discussed later.

The uteri of these animals were not submitted to histological study at the termination of the experiment since they were injected with progestin for only 2 to 3 days and since the uterine motility was studied for several days after discontinuing the progestin. Histological study at that time certainly would not have revealed any proliferation because the animals were not injected long enough to induce good proliferation and any proliferation which may have been induced would have disappeared by the time the motility records were completed. A gross examination of the tissues was made in each case however, and it was found that the uteri were free of adhesions, infections and apparent inflammation. One rabbit (no. 25) noted below developed peritonitis following the perforation of the

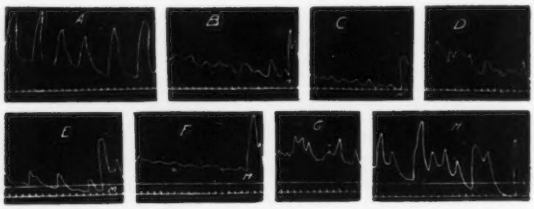

Fig. 1. Records showing the effect of progestin on spontaneous uterine motility in the unanesthetized rabbit. A, normal motility; B, C, D, motility 3, 8 and 12 hours respectively after single subcutaneous injection of progestin (0.2 rabbit unit); E, motility 24 hours after injection, followed by a second and last injection of progestin (0.2 rabbit unit); F, G, H, motility 24, 48 and 72 hours following last injection of progestin. (Rabbit 27, table 1.) $\frac{1}{6}$ size. *M*, mechanical response to demonstrate patency of balloon recording system.

uterus in the course of inserting the balloon for recording. Data from this doe have been considered as reliable until the time when it was reasonably certain that accidental perforation occurred.

Summary. Subcutaneous injection of progestin-containing extracts into non-castrated rabbits whose uteri exhibit normal oestrous motility causes marked reduction in the motility within 24 hours and if the extract is injected for two or three days complete quiescence may be obtained. Normal oestrous motility returns from two to five days after discontinuing the progestin injections.

EFFECT OF THEELIN IN CASTRATED ANIMALS INJECTED WITH PROGESTIN (table 2, fig. 2). In this group of experiments adult female rabbits were ovariectomized 18 to 24 hours after mating with normal bucks. They were, therefore, identical in all respects with those which have been used for the

routine testing of corpus luteum extracts. They were then injected subcutaneously once daily for five days with oily extracts of the corpus luteum which contained standardized amounts of progestin. The first injection of progestin was made on the day of ovariectomy. They were injected with from 1.6 to 5.0 rabbit units total during the period of 5 days. On the fifth day of injections they were injected intravenously in divided doses over a period of 8 hours with the amount of Theelin desired (100–1000 rat units). The uterine motility was recorded immediately before the injection of Theelin, 10 to 12 hours and 24 to 30 hours after the injection of Theelin. It has been amply shown that if motility is to follow injection of Theelin, it will attain maximum amplitude within 24 hours or less of the time of the first injection. After the last recording of motility the animals were autopsied, the ovarian stumps examined to exclude the presence of remnants of the ovary, and the uteri fixed in Bouin's fluid so that histological study could be made subsequently.

In the first six cases of this series the small amount of progestin (1.6 rabbit units in 5 days) sufficed to prevent motility following the intravenous injection of 100–200 rat units of Theelin per kilo of body weight (nos. 1, 2, 3, 4) but this dose was insufficient to prevent normal oestrous motility following the injection of 500 rat units (nos. 5, 6). This is in direct contrast to the situation in the non-progestin-injected castrated rabbit, for in a five day castrate, 2 to 5 rat units of Theelin per kilo invariably bring about marked uterine motility within 24 hours (Reynolds, 1931a). The injection of corpus luteum extracts containing 1.6 rabbit units of progestin therefore increased the refractoriness of the uterus to oestrin from 40 to 100 times. In the remaining animals of this series larger doses of progestin (4.8 to 5.0 rabbit units) and of Theelin were given. The results differ from the first six animals only in that as much as 1000 r.u. per kilo (4300 rat units total in one case) failed to elicit any motility whatsoever. Rabbit 12, which received a total of 3100 rat units (62 cc.) of Theelin intravenously, died from cardiac failure following the final intravenous injection, but previ ous to the time of this last injection no sign of a beginning motility response was observed, yet it may frequently be seen by this time in normal animals treated only with Theelin. It would seem, therefore, that this doe resembles the others of this series. Larger doses of Theelin perhaps might overcome this inhibition but it is technically difficult to give more than this amount. However, the doses given are quite comparable to those which have been found to be inhibited in normal pseudopregnancy (Reynolds, 1931b).

The last point to which attention should be called is the complete inability of corpus luteum extracts in which the progestin has been inactivated by treatment with 2 per cent alcoholic KOH to inhibit Theelin motility. Alkali of this strength, even at room temperature, completely

TABLE 2

Motility response of the uterus of the castrated rabbit treated with progestin, to the intravenous injection of theelin. Controls treated with inactivated progestin

RABBIT	WEIGHT	DAYS POST PARTUM	CASTRATED POST COITUM	AMOUNT OF PROGESTIN ADMINISTERED IN 5 DAYS POST COITUM	AMOUNT OF THEELIN ON DAY 5 POST COITUM	MOTILITY			ENDOMETRIAL PROLIFERATION
						Immediately before theelin	10–12 hours after theelin	24–30 hours after theelin	
	kgm.	*days*	*hours*	*rabbit units*	*rat units per kilogram body weight*				
1	2.9	no recent litter	24	1.6	100	0	0	+ irregular	++
2	2.6	recently pseudo-pregnant	24	1.6	100	0	0	+ irregular	++
3	1.8	2	20	1.6	200	0	0	++ irregular	+
4	2.0	1	20	1.6	200	0	0	+ irregular	+
5	2.1	2	22	1.6	500	0	+ 40″	+++ 40″	++
6	2.1	2	22	1.6	500	0	++ irregular	+++ 40″	++
7	2.1	4	20	4.8	300	0	0	0	+++
8	1.8	2	20	4.8	300	0	0	0	+++
9	2.75	6	24	4.8	500	0	0	0–+ very slight	+++
10	4.0	recently	20	5.0	500	0–+	0	0	++++
11	2.8	no recent litter	20	5.0	1,000	0–+ irregular	0	0	+++
12	3.1	recently	22	5.0	1,000	0	0	see text	++ or +++
13	4.3	6	24	5.0	1,000	+ irregular	0	0–+ irregular	+++

Controls—progestin inactivated in alkalis									
33	2.5	recently pseudo-pregnant	24	2.5	5	0–+ irregular	+++ 50″	++–+++ 50″	0
34	3.0	recently pseudo-pregnant	24	2.5	5	0–+ irregular	+++ 50″	++++ 25″	0
35	3.0	recently pseudo-pregnant	24	4.0	5	+ irregular	+++ 45″–50″	+++–++++ 60″–70″	0

Nos. 1 to 9, treated with crude extract 87 (1 rabbit unit. = 400 mgm.).
Nos. 10–13, treated with purified extract 90.
Nos. 33–35, treated with inactivated crude extract, 92–B(92-A treated with KOH).

inactivates progestin (W. M. Allen, 1930b). Five rat units of Theelin per kilo were sufficient to bring about good motility in animals injected with extracts which previous to inactivation had contained 2.5 to 4.0 rabbit units of progestin (see fig. 2). These animals indicate quite conclusively that the inhibiting factor was destroyed with the proliferating factor (progestin). The question of the possible identity of the inhibitory substance with progestin will be discussed later in the paper.

The histological study of the uteri of these animals showed that in general they were remarkably free of infection. The proliferation of the uteri (see

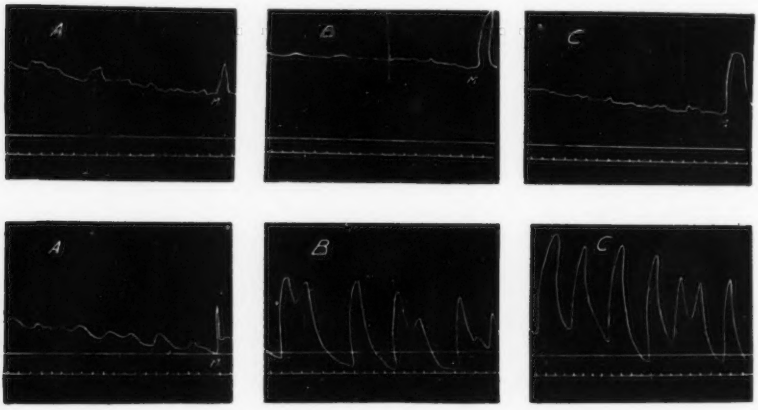

Fig. 2. Records showing inability of Theelin to induce uterine motility in the castrated rabbit after treatment with active progestin (top) and ability to do so after treatment with inactivated progestin (bottom). Top: A, motility after 5 days' treatment with 5 rabbit units of progestin. B and C, motility 12 and 24 hours respectively after 1000 r.u. Theelin per kilo (total, 2800 r.u.). Bottom: A, motility after 5 days' treatment with corpus luteum extract (inactivated by alkali) equivalent to 2.5 rabbit units of active progestin; B and C, motility 11 and 24 hours respectively after 5 r.u. Theelin per kilo (total, 15 r.u.). (Rabbits 11 and 34 respectively, table 2.) $\frac{1}{5}$ size. *M*, as before.

table 2) was not as great as would be obtained in standard test animals injected with similar doses of progestin. These doses (1.6–5.0 rabbit units) should produce complete proliferation in animals castrated 18 to 24 hours after mating and injected over the next 5 days. Such proliferation was not obtained in these animals probably because of the large amounts of Theelin injected on the 5th day. It has been adequately shown that oestrin has a detrimental effect on proliferation and if sufficiently large doses are given, proliferation even in the normal animal can be inhibited completely (Courrier, 1928; W. M. Allen, 1932; Leonard, Hisaw and Fevold, 1932).

TABLE 3

Effect on uterine motility of the daily administration of theelin (200 r.u. daily) during the first five days of pseudopregnancy in the rabbit

RABBIT	WEIGHT	MOTILITY ANTE COITUM	MOTILITY POST COITUM					OVARY DESCRIPTION	ENDOMETRIAL PROLIFERATION
			24 hours	48 hours	3 days	4 days	5 days		
14	1.9	+++ 60″–70″	++ irregular	+–++ irregular	0–+ irregular	0–+	0	7 corpora lutea	++
15	2.1	++–+++ 50″–60″	+ irregular	+ irregular	0	0	0	7 corpora lutea	+
17	4.0	++–+++ 60″–70″	++–+++ 50″–60″	+ irregular	0	0	0	8 corpora lutea	0
18	2.8	+++ 60″–70″	+++–++++ 80″–90″	++–+++ irregular	+ irregular	0–+ irregular	+ irregular	10 corpora lutea	+
19	2.8	++++ 60″–70″	++–+++ 40″–50″	+ irregular	+ irregular	0	0	many corpora	peritonitis
20	1.7	++++ 50″–60″	++++ 60″–80″	+ irregular	0–+ irregular	0–+ irregular	0–+ irregular	7 corpora lutea	+
21	2.5	++ 40″	+++ 50″	+ irregular	0	0	0	9 corpora lutea	+

Controls. Effect on uterine motility of the daily administration of theelin (200 r.u. per day for 5 days) in the normal unmated rabbit

	WEIGHT	BEFORE FIRST INJECTION	AFTER FIRST INJECTION					48 HOURS AFTER LAST INJECTION	
			24 hours	48 hours	3 days	4 days	5 days		
29	2.1	+ irregular	++ 80″–100″	++–+++ 40″–50″	+++ 85″–90″	+++ 50″–60″	+ *see below	+	
30	2.8	+++ 45″–60″	+++ 60″–70″	++++ 60″–70″	+++–++++ irregular	+++–++++ 60″–80″	++++–+++ irregular	++–+++ irregular	
31	2.9	–	+ 20″–30″	+–++ irregular	+++ 60″–70″	++–+++ 60″–90″	++–+++ 60″–90″	++–+++ irregular	
32	5.4	–	+++–++++ 45″	+++–++++ 45″–60″	++++ 45″–60″	++++ 60″	++++ 55″–60″	+++ 80″–90″	

* This animal ovulated during latter part of course of injections.

Summary. Corpus luteum extracts containing progestin (4.8–5.0 rabbit units) will completely prevent any motility response in the castrate rabbit from the intravenous injection of 1000 rat units of Theelin. Extracts containing 1.6 rabbit units of progestin will prevent a motility response from 200 r.u. Theelin per kilo but they will not inhibit 500 r.u. per kilo. Extracts in which the progestin has been inactivated by alkali have no motility-inhibiting effect whatsoever, for a dose of 5 r.u. of Theelin per kilo brings about complete oestrous motility when injected into animals receiving corpus luteum extracts which contained 2.5 to 4.0 rabbit units of progestin before inactivation.

EFFECT OF THEELIN ON UTERINE MOTILITY OF PSEUDOPREGNANT RABBITS (table 3, fig. 3). It has been definitely shown by several investigators that injections of large amounts of oestrin during the first few days after mating will completely prevent the appearance of proliferation but at the same time the development of histologically normal corpora lutea is not prevented (Courrier, 1928; Leonard, Hisaw and Fevold, 1932; W. M. Allen, 1932). We have, therefore, the apparent paradox of histologically normal corpora lutea developing without a concomitant proliferation of the endometrium. This unusual finding gives a method whereby the inhibiting effect of the corpora lutea upon the motility of the uterus may be studied without the presence of proliferation; and at the same time, motility studies may yield direct evidence regarding the functional capacity of these young corpora. Consequently in this series of experiments rabbits were mated and then injected subcutaneously with 200 rat units of Theelin per day for the next five days. The motility of the uterus was recorded daily throughout the period of injections and autopsy was performed the day following the last injection.

The motility data of this series of experiments are interesting in view of the fact that the uterine motility did not persist beyond the first 24 hours (or slightly later in several cases) after ovulation. In other words, despite the absence of corpus luteum effect as determined by the absence of proliferation, there was nevertheless, apparent corpus luteum function as determined by its effect on uterine motility. That this inhibition of motility was due to the corpora lutea present and not to the injection of the Theelin, is shown by the fact that four control rabbits in which coitus was not permitted (and hence in whom corpora lutea were not present) the injection of 200 rat units of Theelin daily for five days was accompanied by marked motility throughout the period of injections.[4] (One doe (no.

[4] A point of difference between the motility in the rabbits of this series and the motility that normally occurs during a similar period of pseudopregnancy in untreated rabbits may be mentioned in passing. In these Theelin-injected rabbits motility persisted through the first twenty-four hours *post coitum*, whereas, normally in the absence of injections of oestrin, the uterus usually becomes quiescent within five to eight hours *post coitum*. (Reynolds and Friedman, 1930; Reynolds, 1932.)

29) of the control group showed feeble motility on the last day of the experiment. It was found that this doe had ovulated several days previously and that there were normal corpora lutea in the ovaries. This occurred despite the fact that complete isolation of the does was maintained, and can only be explained as an instance of spontaneous ovulation.)

The histological studies of the ovaries and uteri of this series of animals revealed that corpora lutea were present in all cases except the controls. These corpora appeared to be histologically normal but the uteri showed little or no proliferation. They are in complete agreement with the series described previously in detail by one of us (W. M. Allen, 1932a). It should be mentioned that no. 19 at autopsy showed an early peritonitis both in gross and upon microscopic study.

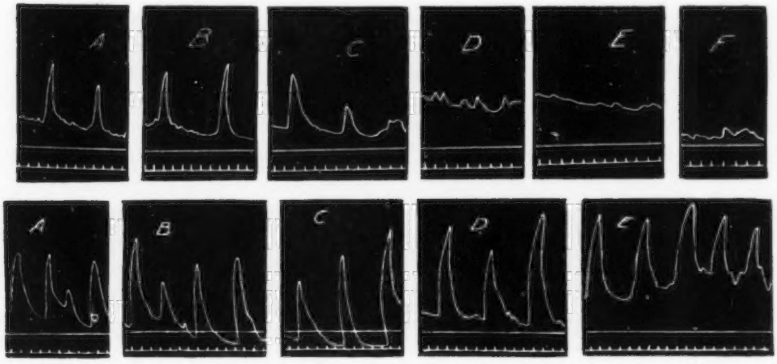

Fig. 3. Records showing uterine motility following coitus (and subsequent ovulation) in the presence of Theelin (200 r.u. daily for 5 days) (top) and uterine motility following injection of Theelin in the absence of ovulation (200 r.u. daily) (bottom). Top: A, normal motility before coitus; B, C, D, E, F, daily motility for first five days of pseudopregnancy in the presence of sufficient Theelin to prevent endometrial proliferation. Bottom: A, B, C, D, E, daily motility following injection of Theelin alone. (Rabbits 18 and 32 respectively, table 3.) $\frac{1}{3}$ size. *M* as before.

The immediate mechanism of this quiescence has not yet been explained, but a recent observation which Markee (1932) had occasion to make in a rabbit with a transplant of uterine tissue to the anterior chamber of the eye suggests that the motility changes we have recorded run *pari passu* with the vascular rhythmic contractions which he has described for the uterine mucosa. Markee found that the rhythmic vascular contractions cease with the vessels in dilatation, at seven hours after coitus. This is indeed a close correspondence in time with the effects we have observed in uterine motility, and probably represents a fundamental interdependence between the two phenomena. It may possibly be that the changes noted above are associated with the 10 to 20 per cent decrease in blood calcium which occurs several hours after coitus in the intact rabbit or after implantation of anterior hypophyseal tissue in intact or castrated rabbits (Hogben and Charles, 1932).

Summary. The subcutaneous injection of 200 r.u. of Theelin per day for the first five days after mating prevents the development of proliferation but does not maintain oestrous motility of the uterus. If, however, the same dose of Theelin is given to unmated rabbits (and, therefore, animals with no corpora lutea in their ovaries) normal oestrous motility is maintained.

Discussion. The observation that daily injection of oestrin (200 r.u. daily for the first five days after mating) prevents the development of proliferation but does not affect the development of histologically normal corpora lutea brings up the question of whether or not the corpora are functional. The previous histological studies of one of us (W. M. Allen, 1932a) give no evidence regarding the activity of these corpora, since no proliferation was produced by them. However, the motility experiments which we have described above indicate directly that these corpora lutea are functional, even though they do not produce proliferation of the endometrium. The uterus becomes quiescent despite the daily injection of 200 r.u. of Theelin, only if corpora lutea are present (table 3). This might be taken as *prima facie* evidence of two hormones (i.e., one which causes proliferation and one which inhibits oestrous motility) since inhibition is obtained without the presence of proliferation. Such an assumption is not warranted, however, because it is quite possible that the daily injection of Theelin has so altered the endometrium that it is unable to become proliferated while under the action of the rabbit's own corpora. This in fact is so, for it has been definitely shown that sufficiently large doses of oestrin will completely inhibit the proliferative capacity of progestin-containing extracts (Tausk, de Fremery and Luchs, 1931; Leonard, Hisaw and Fevold, 1932; W. M. Allen, 1932a). Courrier (1931) also has produced evidence that these corpora are functional. He has found that the continued injection of oestrin for 8 to 10 days after mating is compatible with perivascular changes despite extensive degeneration of the endometrium (and absence of proliferation) provided that corpora are present. We must conclude, therefore, that the corpora lutea under the conditions of these experiments are functional in that they produce either directly or indirectly a hormone or hormones which prevent oestrous motility of the uterus, and which sensitize the endometrium so that perivascular stromal changes may be produced following trauma to the endometrium. They do not prove conclusively that they produce progestin, but it may be supposed that they do because of Courrier's observation that the endometrium is sensitized (assuming that progestin is necessary for the production of the perivascular changes).

The experiments described above also show conclusively that progestin-containing extracts of the corpus luteum contain a substance which inhibits normal oestrous motility and which prevents oestrous motility following the intravenous injection of Theelin. These observations are in apparent

agreement with the extensive experiments of Knaus, who, by studying (Knaus, 1927) *in vitro* responses of the excised uterus to certain drugs, notably pituitrin, has shown that during pseudopregnancy (Knaus, 1930b) the uterus is completely refractory to pituitrin and that near the end of either of these periods this refractoriness disappears. He has demonstrated also that this refractoriness to pituitrin disappears about 24 hours after the excision of the corpora lutea (Knaus, 1930a), and that upon excision of the pregnant horn of a unilateral pregnancy in the latter part of pregnancy (17 days or later) Knaus finds the uterus responsive to pituitrin (1930c), and finally, that corpus luteum extracts (made by the method of Corner and Allen, 1929) bring about a similar refractoriness to pituitrin, even before progestational proliferation is produced. Because of the fact that he can detect this inhibition sooner than proliferation can be fully developed, he has proposed this as a test for progestin (Knaus 1930d, e; 1931). This inhibition to pituitrin appears to be specific, he finds, since the uteri *in vitro* are not refractory to adrenalin or quinine. His evidence indicates therefore that progestin may be the factor responsible for pituitrin inhibition.

A rather different result with this type of experiment has been forthcoming from the work of Siegmund (1930a, 1930b) and Siegmund and Kammerhuber (1931) who applied this technic to the uterus of the mouse, rat and guinea pig respectively. These investigators, working in the same laboratory with Knaus, confirm his findings for the rabbit, but not for these other animals, even though corpora lutea of the oestrus cycle or of pregnancy are present at the time of excision of the uterus. Moreover, Siegmund finds that the uteri of these animals are not affected, as regards the pituitrin response, after the administration of corpus luteum extracts given in oestrus, metoestrus, pregnancy or after castration (Siegmund, 1930c). These corpus luteum extracts were shown to be active by their action on the uterus of the rabbit, in which animal they were standardized according to the method of Knaus, mentioned above. Siegmund, in a separate paper (Siegmund, 1931) discusses the significance of these findings, and is inclined to regard the peculiar response in the rabbit as an attribute of the corpus luteum which is species-specific. Robson and Illingworth (1931), also using the excised uterus, have confirmed Knaus' original finding that corpus luteum extracts contained a pituitrin-inhibiting substance for the uterus of the rabbit, but they have also produced evidence which they interpret as indicating that there may be two hormones, one of which proliferates the endometrium and one which causes refractoriness to pituitrin. They found that some placental extracts (but not all) as well as fluid from an ovarian cyst brought about inhibition of a pituitrin response but did not produce proliferation. These extracts apparently were not assayed for their oestrin content and therefore they may have contained enough oestrin to make the detection of progestin (by the proliferation

reaction) impossible. Better evidence for two hormones is perhaps found in the work of Fevold and Hisaw (1932). They have found that their crystalline corporin in some few instances does not bring about inhibition of the pituitrin response of the excised rabbit's uterus, even though it does produce good proliferation.

We are unable at the present time to offer an adequate explanation of these contradictory findings, but two considerations at least throw some doubt upon their physiological significance. First, the pituitrin response is at best a doubtful criterion of functional activity of the uterus owing to the fact that neither pituitrin nor pitocin has a discernible effect upon the quiescent, non-gravid uterus *in situ* in the unanesthetized rabbit (Reynolds, 1930b). The other consideration is a general one emphasized by van Dyke, Bailey and Bucy (1930), who stress the fact that due regard is not usually given to the importance of the ionic balance of the perfusing medium before and during an experiment in which the excised uterus is employed. Since the publication of their paper this aspect of *in vitro* work with the uterus has not been generally heeded with the meticulous care these investigators prescribe. We must conclude, therefore, that at the present time there is no conclusive evidence that there is a special hormone (differing from progestin) which makes the uterus refractory either to pituitrin *in vitro* or to oestrin *in vivo*. Proof of two hormones can only be obtained by showing that chemically pure progestin does or does not produce both reactions (i.e., proliferation and inhibition of oestrous motility) or that two fractions can be prepared, one of which produces one reaction and not the other, and *vice versa*.

Very little can be said regarding the chemistry of the hormone responsible for inhibition of motility *in vivo*. The fact that the extracts which we have used contain such a substance gives practically no evidence about the chemistry of the hormone, because the discarded fractions were not assayed for their inhibiting power. It is only by assaying all fractions that chemical evidence is attained. It is certain, however, that the active principle is destroyed by treatment with alcoholic potassium hydroxide (2 per cent KOH in 95 per cent alcohol). In this respect the hormone is chemically similar to progestin. If one assumes that the discarded fractions were inactive, then most of the findings known regarding the chemistry of progestin are also applicable to the chemistry of the uterine motility inhibiting hormone, and thus our experiments would appear to favor the view that a single hormone may be involved in these several physiological responses. The special chemical properties of progestin have been discussed recently by one of us (W. M. Allen, 1932b).

SUMMARY

Progestin-containing extracts of the corpora lutea of swine inhibit oestrous motility and prevent motility of the uterus following the intra-

venous injection of Theelin. The hormone responsible for this inhibition is chemically similar to, if not identical with, progestin, in that it is destroyed by alcoholic potassium hydroxide.

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THE HIGH FREQUENCY RESISTANCE OF HUMAN TISSUE

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The extensive use of high frequency currents for heating the deeper tissues of the human body has made it desirable to obtain more information on the path of the current between the electrodes and the distribution of heat in the tissues. The factors which control the current distribution are the relative amounts and positions of the tissues and their specific resistance to the high frequency diathermy current. The earlier work of Nesper (1910) and the more recent work of Philippson (1920) and Hemingway (1931) has shown that the impedance of tissue to alternating current decreases with frequency until high frequencies of diathermy are reached when the impedance to alternating current becomes a pure resistance. At these frequencies the alternating current traversing the tissue will follow the path of the lowest electrical resistance, when possible, and will avoid tissue of a high resistance.

In the measurement of the high frequency electrical resistance of tissues, it is necessary to use alternating current of a frequency of the same order of magnitude as the diathermy frequency, i.e., 500 to 3000 kilocycles per second. With lower frequencies the apparent resistance increases. For this problem we have used a high frequency Wheatstone bridge described in an earlier paper (Hemingway and McClendon, 1932). The alternating current used had a frequency of one million cycles per second and the tissue was balanced in the bridge circuit by means of a capacity in series with a high frequency resistor. It has been shown previously that tissue resistance measured in this manner gives the value of the true high frequency resistance from which the heat production in the tissue can be computed according to the Joule formula.

A possibility mentioned earlier by Wildermuth (1911) is that the high frequency resistance might change on removal of the tissue to the cell for measurement. It is known from the work of Herman (1872), Galeotti (1902), and others that the low frequency resistance (i.e., measured with alternating current of 1000 cycles per second) undergoes large changes on the death of the tissue. However, Hemingway and Collins (1932) have shown recently that although the low frequency resistance of voluntary

muscle undergoes wide variations as the tissue dies, the high frequency resistance remains unchanged for hours, providing the body temperature is maintained. This constancy of high frequency resistance of tissue on death is in agreement with observations of Bachem (1930).

Another possibility which arose was that the cooling of the tissue either after death or on transferring to a conductivity cell might change the high frequency resistance. To investigate this, electrodes were clamped onto a leg muscle of an anesthetized rabbit. The rabbit was placed in the air chamber enclosed by circulating water of any desired temperature mentioned in the earlier paper (Hemingway and Collins, 1932). The rabbit was killed, the body cooled with ice water circulating between the double walls of the chamber, and the temperature again brought back to body temperature. A thermocouple juction, placed on the electrode so as not to interfere with the resistance measurements, recorded the temperature.

TABLE 1

Variation of high frequency tissue resistance with temperature

TIME	TEMPERATURE	RESISTANCE
hours		*ohms*
0	32.6	59
0.5	32.6	59
3.0	21.6	73
7.5	19.2	85
9.5	33.4	56
10.0	32.2	60

The resistance was measured before death and at varying time intervals after death. The electrodes were not disturbed during the experiment. The results are given in the above table, where the elapsed time is measured from the death of the animal. The results prove that although the high frequency resistance of the tissue is increased by cooling, it returns to its former living value on again raising to body temperature. This makes it possible to obtain tissue from a fresh cadaver or from a surgical operation, raise the tissue temperature to body temperature in a conductivity cell, and obtain the true high frequency resistance of the living tissue.

Although it is possible to measure resistance changes due to death or temperature changes with an electrode clamped to living muscle of an animal, it is not possible to obtain specific resistance values of tissues for comparison, by this method, since the cell constant of the systems is not known. To measure specific values of the tissue resistance the conductivity cell, as shown in figure 1, was used. This consists of two telescoping glass tubes with a gold plated electrode at the closed end of the larger

glass tube and another gold plated electrode of the same size on the inserted end of the smaller tube. The tissue is completely enclosed and does not lose water by evaporation while in the bath. The cell constant is computed from the distance between the electrodes and their areas. The value of the specific resistance is

Specific resistance (ohms) = Measured H. F. resistance × Electrode area (cm.) ÷ Distance between electrodes (cm.)

Fat, voluntary muscle, bone, and skin were removed from the living human body during surgical operation. Tissues from the internal organs were obtained from cadavers within two to three hours after death.

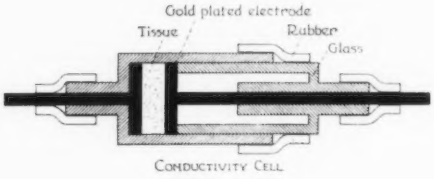

Fig. 1

TABLE 2

High frequency electrical resistance of human tissues (from surgical operations)

TISSUE	SPECIFIC RESISTANCE
	ohms
Skin	289
Fat	2,180
Bone	1,800
Muscle (voluntary)	110

TABLE 3

High frequency electrical resistance of human tissues of internal organs (from fresh post-mortems)

TISSUE	SPECIFIC RESISTANCE
	ohms
Kidney	126
Liver	298
Heart	132
Spleen	256

The averaged results are given in the above tables and are in agreement with results of previous workers using other methods.

Discussion. The relatively high skin temperature in diathermy may be attributed from these results and those of Bachem, to the layer of superficial fat of high resistance which the current must traverse. Bone, a high resistance tissue which can be avoided by the current, would receive little heat energy (except by heat conduction from other tissues). This is in agreement with the temperature distribution of diathermy current made by Schliephake (1929). Once through the skin and superficial fat, the current would follow the muscle tissue and blood wherever possible.

SUMMARY

The high frequency resistances of living human tissues have been measured with a high frequency Wheatstone bridge using alternating currents of one million cycles per second. Evidence is given to show that these values are the resistances of the tissues in situ. The high resistance of superficial fat and of bone explain the electrode heating in diathermy and the small heating of bone.

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THE INSENSIBLE WATER LOSS THROUGH THE SKIN

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According to the usual conception, water may be lost from the surface of the body either as the result of a physical process of diffusion, or as the result of an active secretion of sweat. " . . . . as regards the first kind of loss by evaporation, the skin is apparently behaving as a semipermeable membrane, separating the blood plasma from the external surface of the skin. This membrane is permeable slowly by water, but practically impermeable by inorganic salts such as the sodium chlorid or bicarbonate of the blood plasma." This is the view that Hancock, Whitehouse and Haldane (3) have adopted in their study of the loss of water and salts through the skin.

An examination of the literature indicates that the exact nature of the so-called physical process, generally referred to as insensible perspiration, is not so simple as is often assumed. There is no doubt that an insensible loss of water is constantly taking place from the skin under conditions in which the sweat glands are thought of as being quiescent. But whether this phenomenon is exclusively a function of the epidermal cells or whether it is really dependent upon the activity of the sweat glands is still obscure.

Jürgensen's (4) observations seem to indicate that the insensible perspiration is largely a phenomenon of the sweat glands. Thus he finds that while the sweat glands are in alternate periods of rest and activity, there are always some glands in activity even though their secretion may not be visible, and the skin feels dry and cool. As evidence of this unceasing activity on the part of the sweat glands, Cramer (1), and Schwenkenbecher and Spitta (14) point to the constant presence of salt on the body surface. As the epidermis is impermeable to sodium chlorid, it can come only from the gland orifices.

On the other hand, Loewy and Wechselmann (7) have made the interesting observation that individuals without sweat glands, when exposed in the resting condition to relatively low temperatures, lose as much water by way of the skin as normal persons, but not when either the heat production or

[1] The writer wishes to express her very great appreciation to Dr. A. L. Meyer for his interest and helpful direction during the course of this investigation.

the external temperature was raised. Then an undue elevation of the body temperature was observed. It is Loewy's (6) opinion that the insensible perspiration is determined in large measure by the temperature of the skin and its blood supply.

Other experimental evidence would lead one to suppose that both the sweat glands and the epidermal cells are responsible for the insensible perspiration, but that the elimination from the epidermis is not a passive or physical process, but a definitely controlled physiological process. This appears to be essentially the conclusion that Moog (8) has reached. Atropin, which inhibits the activity of the sweat glands, he finds, diminishes but does not abolish the insensible water loss from the skin. A venous stasis of short duration lowers the temperature of the skin and at the same time reduces the insensible output. On the other hand, a long continued venous stasis actually increases the output above the normal. That a cooling of the skin should be followed by an increase in water elimination can hardly be explained, according to Moog, on purely physical grounds, nor can it be accounted for by an edematous condition of the skin because Schwenkenbecher (13) and Moog (9) failed to establish a consistent increase in clinical cases of edema. Schwenkenbecher (11) attributes the increase under these circumstances to an accumulation of carbon dioxid, which appears to be a reasonable explanation, since it is easily demonstrated that carbon dioxid does stimulate the sweat glands. Moog further makes the rather unexpected observation that neither dermatitis nor the injection of histamine promotes insensible perspiration, although in each case the temperature of the skin as well as its blood supply increases. This, he asserts, is due to the fact that both inflammation and histamine have an injurious effect on the sweat glands.

Now the factors thus far considered, in connection with Moog's work, are conceived as affecting the insensible perspiration by their action on the sweat glands. It is to be noticed that the insensible loss of water is reduced through the agency of either atropin or histamine, but it does not entirely cease. That portion of the insensible moisture of the skin which continues to be eliminated in spite of an abeyance of glandular activity has its origin in the epidermal cells. But this loss, contrary to the usual assumption, is a manifestation of vital activity on the part of the cells themselves. If, for example, one paints the skin with formalin and thereby injures the sweat glands, the insensible perspiration not only continues, but actually increases. Apparently, then, the cells of the epidermis are capable of being stimulated to increased activity and must therefore be regarded as active agents in the phenomenon. Were the imperceptible loss from the epidermal cells a purely physical process, one would expect, says Moog, that as soon as the circulation stopped, it would show a sharp reduction. As a matter of fact, after death the loss of water shows only a

very gradual reduction which seems to be associated with the gradual death of the epidermal cells. It appears to be a part of Moog's conclusion that, although the two sources of insensible perspiration may function simultaneously, they are nevertheless quite independent.

Special staining methods enabled Unna (16) to demonstrate a system of minute canals in the dermis which had their origin at the apices of the papillae, and after spreading in radial fashion toward the surface layers, returned to the interpapillary region and finally ended in the ducts of the sweat glands. Obviously such an arrangement makes possible the evaporation of water at the orifices of the sweat glands which is not the product of an active secretion.

According to Frieboes (2) the passage of water through the epidermis is not wholly confined to the canalicular structure but is effected in large measure by a meshwork of fibers having hydrophilic properties. This is somewhat akin to the idea advanced by Rothman (10), namely, that during the process of keratinization water, which had previously constituted an integral part of the cell protoplasm as bound water or water of hydration, becomes free to undergo evaporation.

METHOD. *Phosphorus pentoxid method.* Anhydrous phosphoric acid (phosphorus pentoxid) is an excellent hygroscopic agent and was found eminently suitable for the absorption of water vapor from the skin. In order to give the powder greater surface, it was mixed with pumice previously heated to white heat by means of a blowpipe to remove any possible traces of organic matter. Two sets of U tubes were used. One collected water vapor from a given area of the skin at the same time that the other collected water vapor present in the room air. Each set of tubes was connected to a wet meter, and each meter connected to a negative pressure outlet by means of which the air flow could be regulated at will.

In determining the quantity of water vapor present in the room air, the air was drawn directly into the set of U tubes where it lost its moisture, and then through the meter where the volume of air was measured. In determining the quantity of water vapor given off by the skin, however, the room air was first allowed to pass through a glass applicator placed over a small area of the skin, and then into the U tubes containing the phosphorus pentoxid. The rubber tubes which carried the room air to both sets of U tubes were brought close together with adhesive tape in order that the air going to both sets of U tubes should be identical in composition. Thus, the difference between the quantity of water vapor collected over the skin in a given time (which also included the water vapor present in the room air), and the quantity of water vapor present in the same volume of room air, constitutes the amount of insensible perspiration that must have been given off by that particular area of the skin.

The same sets of U tubes could be used about forty times without being

changed. In the last experiment of one series in which the tubes had been used for thirty-nine experiments, the amount of moisture which passed over into the second tube of one set was 0.0009 gram, and of the other set 0.0006 gram. This gives some idea of the efficiency of the method. The glass applicator had a diameter of 5 cm. which means that it covered a skin area of approximately 19.6 sq. cm. Although the observations have been confined chiefly to two areas, namely, the left cheek and the left supramammillary region, they clearly indicate that the rate of insensible perspiration from these areas is definitely modified by certain changes in the physical condition of the air, and that the process is quite independent of any demonstrable activity on the part of the sweat glands.

It is possible that the quantity of vapor found might depend upon the rate of its removal from the enclosure. When the air is unsaturated, the evaporation of sweat, as is well known, is much more rapid in moving air than in still air. While it appears to be a characteristic of the insensible perspiration that the supply of water keeps pace with its evaporation, it is conceivable, nevertheless, that a slight excess of moisture might be present on the skin from time to time which would easily escape observation. The rate of air flow most commonly employed in these experiments lay between 0.3 and 0.5 cubic foot per half-hour. This means that the air of the enclosure was being changed at the rate of about three to five times per minute. Variations within these fairly wide limits had no measurable effect on the amount of moisture collected. When, however, the flow was definitely above these limits, e.g., 0.6 to 0.7 cubic foot per half-hour, there was a very noticeable increase in the elimination of moisture. Since the vapor tension of the room air as it enters the enclosure must vary inversely with the rate of air flow, and since the phenomenon that we are considering is one of evaporation which is determined by the vapor tension of the air, the insensible perspiration would be expected to bear a definite relationship to the current through the enclosure. As a matter of fact, there appears to be a critical velocity below which the elimination is practically independent of air velocity and above which the elimination increases with the rate of air flow. It is clear, however, that at the lower rates of air flow more water is available for evaporation than is actually evaporated.

Silver nitrate method. According to Schwenkenbecher (12), sodium chlorid is eliminated from the human skin exclusively through the activity of the sweat glands, and the epidermis is impermeable to an aqueous solution of sodium chlorid. It is generally held that sweat is a salt solution which is hypotonic with reference to the blood plasma (C. Kittsteiner, 5). If this is true, then the demonstration of salt on the skin surface may be accepted as evidence of activity on the part of the sweat glands. In order to avoid mistaking former activity for present activity, the area under observation was always freed from any possible traces of salt by gently

washing the area with distilled water a few minutes before the experiment began. The method of revealing the presence of salt is extremely simple. When the temperature of the room was sufficiently high to cause sweat secretion, a piece of smooth white paper applied firmly against the skin for a few moments would, upon being immersed in a ten per cent silver nitrate bath and then exposed to the sunlight, develop minute punctate areas of a deep brownish color. These punctiform areas indicate the position of active sweat glands and become more numerous as the temperature of the room rises. The method is sufficiently delicate to detect the presence of salt in a concentration as low as 0.005 per cent. Throughout this investigation there was not a single instance when evidence of insensible perspiration was not obtained, and only five instances of sweat secretion. In each of these five cases the elimination rose from 18 to 72 per cent above the average, and the silver nitrate test showed a positive reaction.

The experiments on the subject were made in the sitting position. All the experiments were carried out in a special room. When constant atmospheric conditions other than those naturally existing were required, air was supplied by a conditioning apparatus. Care was taken to place the applicator over the same skin area each time, and the silver nitrate test was applied immediately after each experiment.

EXPERIMENTAL RESULTS. *Effect of humidity.* If water appears as the result of diffusion through the skin acting as a semipermeable membrane, it must be present in the free state. Its evaporation would therefore be determined by the temperature of the skin and would continue until the air in contact with the skin became saturated. It is probable, however, that the process ceases some time before the saturation point is reached. This seems to follow from experiments with cobaltous chlorid. If discs of paper impregnated with cobaltous chlorid are placed within the enclosure, the amount of moisture absorbed falls considerably short of the amount collected under similar conditions by the phosphorus pentoxid method. At the end of a short period, say five minutes, the gain in weight of the cobaltous chlorid may agree very closely with the gain in weight of the phosphorus pentoxid, but at the end of half an hour the discrepancy becomes very great. This is not because the capacity of the paper is limited to the amount of water absorbed, for a considerably greater absorption will be found to have taken place if the paper be placed over water for half an hour, even though the water has a lower temperature than that which prevails in the enclosure. Now the aqueous tension of the hexahydrate of cobaltous chlorid has never been determined, but is probably of the magnitude of that shown by the hexahydrate of strontium chlorid or the pentahydrate of cupric sulphate. At 30°C. these salts exert a tension of about 12 mm. Hg as compared to 31.5 mm. Hg, the tension shown by water. The inference from these experiments appears to be that we are

dealing not with water having its origin in a process of diffusion, but with water of hydration.

This inference finds additional support in another group of experiments. If, instead of passing room air through the enclosure over the skin, we pass dry air, the mass of water vapor collected is always greatly increased, in some cases by as much as 100 per cent or more. This means that a relative humidity of approximately 20 per cent (one commonly employed in these experiments) suffices to reduce the vaporization 50 per cent or more. At this rate we would expect, in conformity with our previous argument, that

Effect of passing dry air—cheek

DATE (1931)	DRY BULB	WET BULB	REL. HUM.	EFF. TEMP.	VAP. TEN.	ELIMINATION	AIR VOL.
			Control				
	°F.	*°F.*	*per cent*	*°F.*	*inch/Hg*	*grams/hour*	*cu. ft./hr.*
March 16	77.0	56.5	25.0	69.0	0.228	0.0782	0.930
			After passing dry air				
March 16	78.5	57.0	23.0	70.0	0.223	0.1536	0.908
			Control				
March 31	77.5	56.5	25.0	69.3	0.237	0.0712	0.808
			After passing dry air				
March 31	78.0	56.5	23.0	69.6	0.219	0.1292	0.736

Effect of passing dry air—chest

DATE (1931)	DRY BULB	WET BULB	REL. HUM.	EFF. TEMP.	VAP. TEN.	ELIMINATION	AIR VOL.
			Control				
	°F.	*°F.*	*per cent*	*°F.*	*inch/Hg*	*grams/hour*	*cu. ft./hr.*
March 6	70.0	50.0	19.0	63.8	0.136	0.0268	0.690
			After passing dry air				
March 6	70.5	50.0	17.5	64.1	0.130	0.0556	0.812
			Control				
March 17	72.5	53.0	23.5	65.8	0.187	0.0204	0.774
			After passing dry air				
March 17	73.0	53.0	22.0	66.1	0.180	0.0504	0.682

the insensible perspiration would cease at a relative humidity of about 60 per cent. That this is actually the case was demonstrated by several experiments in which air at 70 per cent saturation was passed through the enclosure. Not only did vaporization stop, but the air itself on coming in contact with the skin lost moisture, indicating that a reversal of the process had taken place. (See figures on page 65.)

By way of further investigation of this particular point, the entire body instead of only a limited area of the body surface was exposed to gradually increasing relative humidities. It was now observed that when the humidity reached 60 to 70 per cent of saturation, the vaporization of insensible perspiration was diminished, and that a further rise in the relative humidity up to nearly 80 per cent actually caused a return of the amount of evaporated moisture to the normal level.

Effect of increasing relative humidity—cheek

DATE (1932)	DRY BULB	WET BULB	REL. HUM.	EFF. TEMP.	VAP. TEN.	ELIMINATION	AIR VOL.
	°F.	°F.	per cent	°F.	inch/Hg	grams/hour	cu. ft./hr.
Feb. 1	72.0	59.0	45.0	67.3	0.360	0.0668	0.852
Feb. 1	71.0	60.0	52.0	67.0	0.387	0.0658	0.872
Feb. 1	73.5	65.0	63.0	70.0	0.527	0.0570	0.820
Feb. 1	75.0	69.0	74.0	72.2	0.638	0.0580	0.832
Feb. 1	77.0	72.0	79.0	74.5	0.732	0.0800	0.832
Feb. 18	74.0	60.0	43.0	68.7	0.360	0.0798	0.824
Feb. 18	74.5	65.0	60.0	70.7	0.508	0.0630	0.848
Feb. 18	75.0	68.0	70.0	71.9	0.595	0.0604	0.844
Feb. 18	76.5	72.5	82.5	74.5	0.732	0.0726	0.854
Feb. 25	72.0	62.0	57.0	68.1	0.448	0.0606	0.844
Feb. 25	73.0	64.0	61.0	69.4	0.499	0.0644	0.826
Feb. 25	75.0	68.0	70.0	71.9	0.595	0.0558	0.810
Feb. 25	75.5	69.5	74.0	72.8	0.650	0.0784	0.812
Feb. 25	77.5	72.5	79.0	75.0	0.746	0.0778*	0.824
Mar. 3	73.0	63.0	57.0	69.0	0.465	0.0492	0.808
Mar. 3	74.0	66.0	65.0	70.7	0.555	0.0556	0.802
Mar. 3	75.0	68.5	72.0	72.0	0.616	0.0584	0.814
Mar. 3	78.0	73.5	81.0	75.8	0.783	0.0654	0.848
Mar. 3	78.0	74.5	85.0	76.1	0.810	0.0636	0.828

* Silver nitrate test showed sweating.

Discussion. The present research was undertaken primarily for the purpose of throwing additional light on the nature of the process of insensible perspiration. The literature thus far has not made it clear whether this process occurs independently of the sweat glands or whether it really constitutes another phase of their secretory function. If the process is one

occurring quite apart from any activity on the part of the sweat glands, there still remains the question whether it is simply a matter of physical diffusion, governed solely by environmental factors, or whether it is an active phenomenon under physiological control.

During the course of this investigation numerous instances of the insensible loss of water from the skin have been observed in which the silver nitrate method failed to reveal the presence of salt. Unless the sweat glands are regarded as being capable of secreting a fluid entirely devoid of salt or containing salt in mere traces, these instances afford definite evidence that water having its source in the epidermal cells and not in the sweat glands is constantly being lost by evaporation. The attempt which is sometimes made to base such an opinion upon the fact that persons born without sweat glands may, nevertheless, lose as much water from the body surface as normal persons under ordinary temperature conditions, is hardly beyond criticism, for it is perfectly conceivable that an abnormality in respect to the sweat glands may involve an abnormality in respect to the insensible perspiration also. In other words, the capacity of losing water in the absence of sweat glands may have resulted as a compensation and may not exist in the normal individual.

If the insensible perspiration be regarded as a phenomenon unconnected with the sweat gland function, one may think of it as a physical process in which the skin, serving as a semipermeable membrane, permits the outward diffusion of pure or nearly pure water. According to this view, which is the one accepted by Hancock, Whitehouse and Haldane (3), the loss of water by evaporation would be expected to vary in a manner determined by the operation of certain simple physical laws. Some of the results of the present investigation are consistent with such a theory. This is true, for instance, of the effect of changes in atmospheric temperature. It also appears to be true of the effect of air currents when acting upon the body as a whole. However, when the influence of humidity is considered, the physical theory in this particular form does not suffice to explain the phenomena actually observed. The exposure of a relatively small area of skin to perfectly dry air causes the loss of water by evaporation to increase 100 per cent or more above the value found when the air has a relative humidity of only 20 per cent. When the same area is brought in contact with air having a relative humidity of approximately 70 per cent, evaporation ceases. Such a result is not consistent with the idea that the skin acts as a semipermeable membrane, and leads one to suspect that water is not present in the free state, but rather in a state in which its tension is considerably less than that of free water.

But there is still another peculiarity in the behavior of the insensible perspiration with respect to atmospheric humidity and one which makes it very doubtful whether we are dealing with a process essentially physical

in nature at all. In general, the initial effect of increasing the water vapor content of the air of a room, at temperatures which do not produce sweating, is to diminish the amount of evaporation from the skin; but with a still further increase in the water vapor content of the air, the evaporation from the skin begins to increase and returns finally to the normal level or may in fact exceed this level. The implication of this result appears to be that the skin is not acting as a physical entity, but rather as a vital structure possessing the inherent capacity of increasing its water output in spite of a gradually increasing humidity of the surrounding air. It may be of interest to remark incidentally that the beginning of this upward trend in the elimination was associated with the onset of an uncomfortable subjective sense of warmth.

By way of criticism it may be argued that owing to the difficulty of evaporation from the general body surface when the entire body is subjected to an increasing atmospheric humidity, the small area immediately under observation becomes richer in water either as the result of diffusion from adjacent areas or as the result of a local change in the state of the capillaries; and, since the movement of air through the applicator would favor an escape of moisture, any local increase in insensible perspiration could be attributed to the experimental conditions and would therefore not necessarily have any real physiological significance. As a matter of fact, however, the rate of air change in the applicator is no greater and probably considerably less than the rate of air change between the skin and clothing due to convection currents. It will also be recalled that there is always more water available for evaporation in a given area of skin than is actually evaporated. It is not easy to understand how, under such circumstances, any additional water could possibly result in an increased vaporization. That it is due to an increase in atmospheric temperature is hardly likely because an increase of 5°F. in the room temperature would be expected to increase the skin temperature relatively, but slightly. The vapor tension of the water in the skin would also increase slightly. But the vapor tension of the room air, by increasing the relative humidity to 80 per cent, has increased out of all proportion to the increase in vapor tension of the water in the skin. In spite of this the skin continues to increase its water output against a greatly increased vapor tension of the surrounding air. In view of the observation that an increasing humidification of the atmosphere does tend to diminish the insensible perspiration as measured locally, but that this initial effect is followed by a definite increase and that this appears to be coincident with the onset of discomfort, the evidence strongly suggests an active intervention on the part of a structure constituting the immediate source of the water undergoing vaporization.

We seem to be led to the following position. The water which by its evaporation constitutes the insensible perspiration is bound in such a

manner as to materially reduce its tension below that of water in the free state; but the water thus bound may under certain circumstances be released by the exercise of a vital function on the part of the binding structure. The exact locus of this physiological activity cannot be said to have been determined with certainty. Since this activity occurs without the demonstrable presence of sweat, one is strongly tempted to believe that it takes place in the epidermal cells or structures in the epidermis other than the sweat glands, although the possibility still exists that the sweat glands may secrete a fluid of very low salt content some time before the appearance of true sweat. But a possible activity of this sort could hardly be interpreted as secretory in nature because that would involve the evaporation of pure water.

SUMMARY

1. Throughout this investigation there was not a single instance when evidence of insensible perspiration was not obtained, and only five instances of sweat secretion, in each of which the elimination rose from 18 to 72 per cent above the average and the silver nitrate test showed a positive reaction, indicating that a totally different mechanism had come into play. It seems probable, therefore, that the two processes are quite independent.

2. This work has tended to show that water exists in the skin as bound water or water of hydration according to the theories of Frieboes and Rothman, and not as free water as must be inferred from the theory of Hancock, Whitehouse and Haldane.

3. The evidence also suggests that the process is really a vital one under definite physiological control when the effect of humidity on the entire body is considered. Then the skin acts as a vital structure possessing the inherent capacity of increasing its water output in spite of a gradually increasing humidity of the surrounding air.

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SIMULTANEOUS INTERNAL AND EXTERNAL STIMULATION OF THE IRIS BY ADRENIN

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The radial dilatation of the iris caused by adrenin, when the organ has been sensitized by removal of the superior cervical ganglion, has been studied under various conditions. The phenomenon appears after subcutaneous injection of adrenalin or its conjunctival instillation in rabbits (Meltzer and Meltzer-Auer, 1904) and after splanchnic stimulation or emotional excitement in cats (Elliott, 1912; Hartman, McCordock and Loder, 1923) or asphyxia (Kellaway, 1919). Under the same conditions it does not appear in the other eye, if that eye is left normally innervated. Both Elliott and Hartman and his collaborators noted a slight residuum of the phenomenon on exciting their animals when the adrenal glands were inactivated, a result which Cannon and Bacq (1931) attributed to the action of sympathin. It is clear that by proper sensitization of the iris an extra amount of circulating adrenin, whether injected or secreted, can have an effect which is not otherwise manifest. It seemed possible that by instillation of adrenalin into the conjunctival sac, the iris, though not obviously affected, might be sensitized, and that then a greater concentration of adrenin in the blood would become effective. If this should prove true, a simple method of studying the conditions which cause increased adrenal secretion would be available. For that reason the present study was undertaken.

METHOD. Cats were used. The superior cervical sympathetic ganglia were removed aseptically under ether anesthesia.

The observations were made in a dark room with the pupils illuminated by means of an electric lamp placed at a fixed distance (60 cm.) from the animal's eyes. The changes in the pupil and in the nictitating membrane were determined by direct measurement, made by holding a millimeter scale close in front of the eyes. The animals became accustomed to the procedure after some time. In standard conditions the readings were practically constant for each animal.

RESULTS. After the operation the animals were artificially angered (movements, showing a dog). Under these circumstances there appears

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a strong dilatation of the pupils which recedes sharply a few seconds after the end of the stimulation, leaving frequently a more moderate widening which disappears gradually after a variable time (1 to 3 minutes). This latter effect is more marked the stronger the emotional excitement. There occurs also a marked contraction of the nictitating membrane which lasts from 2 to 5 minutes.

During the first days (about 15) that follow the operation, if 2 to 4 drops of adrenalin 1:10,000 are instilled in one conjunctival sac (using the other side for control), there occurs immediately a contraction of the corresponding nictitating membrane, whereas there is no perceptible change in the pupil.

At about this time (15 days after the operation) a similar instillation of adrenalin begins to produce a dilatation of the pupil. As time passes this dilatation is earlier, more marked and longer.

When adrenalin is instilled in one eye and after 10 minutes or longer the animal is excited emotionally, there appears, as stated before, a dilatation of both pupils. The instilled one shows a more intense and lasting widening (5 to 10 minutes). If several of these emotional excitements are produced at short intervals (5 to 10 minutes) there occurs on the instilled side a much longer dilatation (3 to 4 hours) (see fig. 1, A).

Dilutions of adrenalin varying between 1:1,000 and 1:50,000 were used for the instillations. The stronger the solution, the earlier, the more marked and the more lasting the effects. All the results mentioned were obtained after using the 1:10,000 solution.

If the animal is emotionally excited some hours (3 to 8) after the instillation of adrenalin, when the effects of the instillation have apparently disappeared (both pupils having the same transverse diameter), there still occurs a difference in the consequent dilatation in favor of the instilled eye (see fig. 1, B). Removal of one adrenal and denervation of the other one (two cases) or removal of both adrenals (one case) diminish the intensity of all the above-mentioned phenomena but do not suppress them.

Intravenous injection of adrenalin, after instillation of adrenalin in one eye, produces a larger and more persistent pupillary widening in the instilled eye (see fig. 1, C).

Experiments were tried in normal cats without removal of the superior cervical ganglia, using doses of adrenalin similar to those mentioned above, and the results were negative. However, if strong solutions are instilled for a sufficiently long time (15 to 30 minutes) some effect appears (dilatation or contraction).

Discussion. When the superior cervical sympathetic is removed, the iris is still innervated by the third cranial nerve and, as Cannon (1915) remarked, the instantaneous dilatation of the denervated pupil in emotional excitement is explicable as due to central inhibition of the tonically active constrictor impulses.

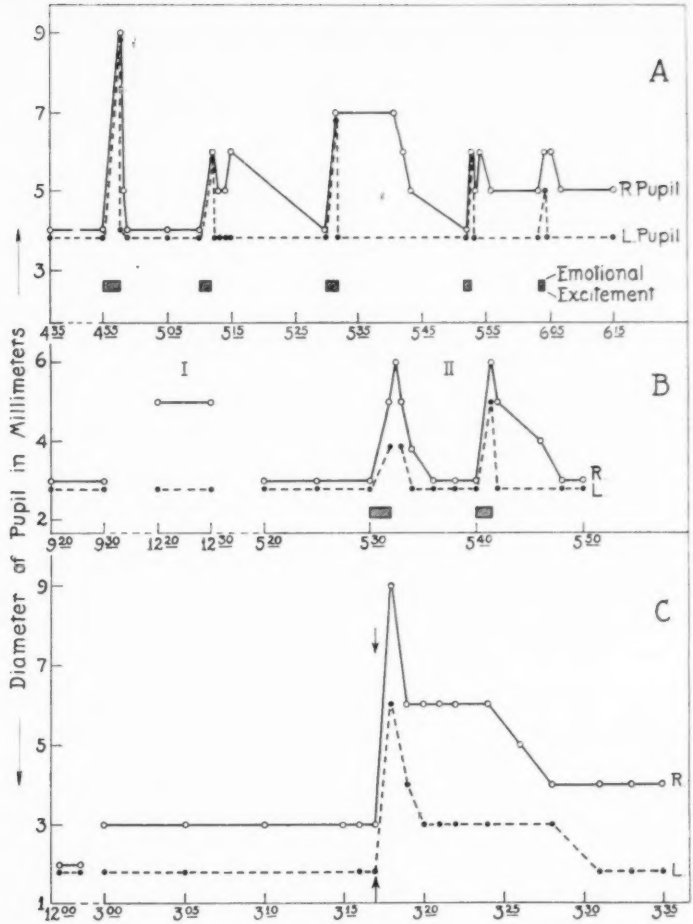

Fig. 1. Cats, with both superior cervical sympathetic ganglia removed. Continuous lines show the diameter of the right pupil, dash lines the diameter of the left pupil.

A. Adrenalin 1:10,000 instilled in right eye at 4:35. At 4:55, the animal was excited emotionally and there appeared a dilatation of both pupils, after which they recovered their previous dimensions. At 5:12 the animal was excited again. In the non-instilled eye the pupil recovered almost instantly, whereas the instilled one showed a more intense and lasting widening. At 5:30 another emotional excitement caused the same effect. At 5:52, after repeated production of emotional excitement, the pupil in the instilled eye was dilated for a few hours.

B. Adrenalin 1:10,000 instilled in right eye at 9:30. I shows the effect three hours after the instillation. II shows the different response of the instilled eye to emotional excitement after the apparent effect of the adrenalin had disappeared.

C. Right pupil is dilated from previous instillation of adrenalin at 12:05. At 3:19 an intravenous injection of adrenalin produced a much more marked and longer effect in the right pupil.

The smaller dilatation persisting after the emotional stimulation has ceased is due to adrenal secretion (Elliott and Kellaway, loc. cit.).

The negative effects on the iris on instillation of adrenalin during the first days after the denervation are due to lack of sensitization. At this stage the nictitating membrane already reacts.

The increased reactions of the pupil to emotional excitement after adrenalin has been instilled might be explained as a summation of effects. This hypothesis would also account for the more prolonged dilatation obtained in the instilled eye after repeated emotional excitement. This explanation, however, is apparently not admissible since it does not cover the more marked widening obtained several hours after the effect of the instillation has ceased and the pupils are equal (fig. 1, B). The great instability of adrenalin, especially when in contact with the tissues, is likewise not compatible with the idea of a slow absorption. Furthermore, the amount of adrenalin instilled (two drops of a 1:10,000 solution) is probably too small to render possible its action several hours after the instillation.

The only other hypothesis satisfactorily accounting for the facts is a sensitization of the iris on instillation. This theory, first proposed by Stuber, Russmann and Pröbsting (1923) would account for all of our results.

SUMMARY

The reactions of the iris, denervated by removal of the superior cervical sympathetic ganglia, to adrenalin instilled in the conjunctival sac and to emotional excitement were studied in cats.

Instillation of adrenalin in the conjunctival sac begins to produce a marked dilatation of the corresponding pupil only about 15 days after the denervation. As time passes this dilatation occurs earlier, is more marked and lasts longer. Emotional excitement produces stronger and longer pupillary widening of the previously instilled eye (see fig. 1a). On repetition of emotional excitement the effects increase. These reactions occur even several hours (3 to 8) after the instillation, when both pupils are apparently in identical conditions (see fig. 1b).

Inactivation of the adrenals greatly diminishes but does not wholly suppress the above-mentioned results.

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FLEXOR RIGIDITY OF THE HIND LEGS AND PRIAPISM IN THE "SECONDARY" SPINAL PREPARATION OF THE MALE CAT

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In the course of recent experiments a spinal preparation with as little "shock" as possible was required. It occurred to us that this purpose might be attained by first decerebrating and then decapitating the animal.

Such a "secondary" decapitate preparation, as we shall call it for convenience, shows an entirely different pattern from that of the ordinary decapitate animal (Sherrington, 1909). In the latter preparation the muscles present no special distribution of "tone" and the limbs remain in every position given to them. In contrast to this picture, the secondary decapitate preparation exhibits strong, persistent, springlike *flexor rigidity* in the hindlimbs, usually associated with marked *priapism* in the male. As this syndrome has not yet been described, so far as we know, a brief report of these experiments may be presented.

There are a few statements in the literature concerning the effect of low spinal transection after decerebration on the flexion reflex of an isolated flexor muscle in the cat (Sherrington and Sowton; Sassa and Sherrington; Forbes, Cobb and Cattell; Forbes, Barbeau and Rice; Gerard and Forbes). All these investigators report that the flexion reflex in such a preparation is slightly augmented, but on the other hand characterized by a marked diminution or an abolition of after-discharge.

In view of these statements the sustained flexor pattern observed in the experiments of this series was the more striking to us.

METHOD. All experiments were performed on the cat.

Under profound anesthesia induced by ether, a tracheal cannula was inserted, both carotids were ligated and a string was passed around the vertebral column for later ligation of the vertebral arteries according to the method devised by Sherrington. The atlanto-occipital membrane was then exposed. After freely opening the cranial cavity and the dura mater an intercollicular transection of the brain-stem was performed with a blunt spatula across the opening in the bony tentorium. The whole brain in front of this transection was then removed. In some of the experiments to minimize hemorrhage the vertebral arteries were compressed immediately prior to the severance of the brain-stem.

TABLE 1

Results of secondary spinal transection after primary decerebration

NUMBER OF EXPERIMENT	ONSET OF F. R.	FLEXOR RIGIDITY (F. R.)	PRIAPISM	REMARKS
1	Immediate	+	In the first 7 experiments no annotations about priapism	
2	Immediate	++++		
3		−		
4	Immediate	+++		
5	Immediate	+		F. R. lasting only a few minutes
6		−		
7	Immediate	+++		
8	Immediate	+++	++	F. R. present for $5\frac{3}{4}$ hrs.
9	Immediate	++++	++	F. R. present for 4 hrs.
10	Immediate	++++	++	F. R. for 14 hrs.
11	Immediate	++++	+++	F. R. for 23 hrs.
13	Immediate	+++	++	
14	Immediate	++++	++++	Some adductor and flexor rigidity in frontlegs
17	Immediate	++++	++	
18	After 12′	++	++++	F. R. not typically springlike
19	Immediate	+++	+++	F. R. strong for 3 hrs., stronger in hindleg with stronger D. R.
20	Immediate	++++	++++	Maximal F. R. for 12 hrs.
21	After 20′	++++	++	Onset of F. R. probably delayed because condition of animal was rather poor immediately after decapitation F. R. for $9\frac{1}{2}$ hrs.
22	Immediate	+++	+++	F. R. maximal in hindleg with stronger D. R.
23	Immediate	++++	+	F. R. abolished by section of posterior roots caudad of L. I.
24	After 1 hr. 20′	+++	+++	F. R. for 5 hrs. At first F. R. stronger in hindleg with *weaker* D. R. After 1 hr. 30′ stronger in hindleg with *stronger* D. R. Augmentation of F. R. after tertiary spinal transection at L. I. Abolished after section of posterior roots from L. I. on downwards
28		−	−	After decapitation (6 mm. below calamus) persistence of D. R. in hindlegs for 4 minutes
29	After 1 hr.	++	−	
30	Immediate	++	++++	F. R. stronger in hindleg with stronger D. R. Augmentation of F. R. after tertiary transection at L. I.

TABLE 1—*Continued*

NUMBER OF EXPERIMENT	ONSET OF F. R.	FLEXOR RIGIDITY (F. R.)	PRIAPISM	REMARKS
31	Immediate	++	++	F. R. stronger in hindleg with stronger D. R.
32	After 1 hr.	++	+	Condition of preparation rather poor all through experiment. Tertiary spinal transection at L. I. did not result in augmenta- of F. R.
33	Immediate	++	++	F. R. for 7 hrs. Stronger in hindleg with stronger D. R. Very strong F. R. and maximal priapism after tertiary spinal transection at L. I.
34	Immediate	+++	+	F. R. for 10 hrs. Augmentation of F. R. after tertiary spinal transection at L. I.; abolished after section of posterior roots
35	Immediate	++	++	Augmentation of F.R. after tertiary spinal transaction at L. I. Abolished by section of posterior roots.
36	Immediate	+++	++	Increase of F. R. after transection at L. I.
38	Immediate	++	+?	Augmentation of F. R and priapism after spinal transection at L. I.
47	Immediate	++	+	Increase of F. R. and priapism after spinal transection at L. I.
65	Immediate	+++	−	F. R. for at least 7 hrs. Augmentation of F. R. after tertiary spinal transection at L. I.
67	Immediate	++	+	Slight adductor tone in frontlegs, appearing 5 hrs. after decapitation. F. R. increased after tertiary spinal transection at L. I. F. R. observed for 12 hrs.
(73)	Immediate	++	+?	Secondary section at L. I. Increased D. R. of front legs. Decrease of F. R. in right leg after skinning. F. R. observed for 6 hrs.
(74)	Immediate	+++	+	Secondary section at L. I. Immediate increase of D. R. of front legs. F. R. observed for 9 hrs. Decrease in F. R. of right leg after skinning. Partial recovery after a few hours

TABLE 1—*Concluded*

NUMBER OF EXPERIMENT	ONSET OF F. R.	FLEXOR RIGIDITY (F. R.)	PRIAPISM	REMARKS
75	Immediate	+++	+	*Left adrenalectomy.* Increase in F. R. of right leg after tertiary spinal transection
76	98′	++	++++	Bilateral adrenalectomy
77	80′	++	++++	Bilateral adrenalectomy
78	8′	++	++++	Bilateral adrenalectomy
79	Immediate	++	++++	Bilateral adrenalectomy after brain sections
85	Immediate	++	++	F. R. observed for 4 hrs. Animal sacrificed
86	Immediate	++	++++	F. R. observed for 4.5 hrs. Animal sacrificed
87	Immediate	++	++++	F. R. observed for 3 hrs. Animal sacrificed

When decerebrate rigidity had appeared in the front legs or in all four limbs, the string around the vertebrals was pulled tight, the atlanto-occipital membrane was incised and the "secondary" decapitation performed. The level of this latter transection varied somewhat in the different experiments, the plane of the section being usually from 1 to 5 mm. caudal of the calamus scriptorius. Artificial ventilation and heating were started after the decerebration. In the experiments with secondary low spinal transection this operation was performed at the level of the first lumbar segment.

RESULTS. Such a secondary decapitation (see table 1) results not only in the disappearance of decerebrate rigidity, as might be expected since the primary decapitate preparation does not show this rigidity, but in the replacement of this extensor tone by a marked, sustained flexion posture, flexor rigidity (F. R.) of the hindlegs. This phenomenon usually appears immediately after the second transection, sometimes following an interval varying from a few minutes to a few hours. Apparently this latency in appearance of F. R. is short when the condition of the animal is good, as may be evaluated from the prompt establishment of strong decerebrate rigidity after the decerebration. If the onset of this extensor tone is delayed for some reason or another (too deep anesthesia, loss of blood, etc.) the F. R. sets in after a longer interval and may be only moderately developed.

With well established flexor rigidity, strong force must be exerted to overcome the flexion of these limbs, and, as soon as they are released, the

hindlegs return into flexion with a quick, springlike movement (fig. 1). In preparations with marked F. R. the tension required to extend the legs may be from 1300 to 1500 grams or higher. In one animal a tension of 2500 grams was necessary to extend the leg.

It is, however, possible to overcome this tendency to flexion by continuing the extension of a hindleg for 10 to 15 seconds. If after such a sustained passive extension the leg is gently released, it may remain extended or slowly return to the flexed position. If the leg remains extended, a slight stimulus applied to it, however, is sufficient to evoke prompt and lasting flexion.

The flexion is most marked in the hip and knee joints, less in the ankle joints, although in some instances marked dorsiflexion of the feet was observed. The superficial flexor muscles of the hip on the lateral side of the thigh, especially tensor fasciae femoris, and also the lateral knee flexors, may show fascicular twitchings, which often become more pronounced on passive extension of the leg.

The intensity of this F. R. is sometimes not equally strong in both hindlegs, but more pronounced on one side than the other. So far as our experience at present goes, we have nearly always observed that this occurs when the decerebrate rigidity after the primary decerebration was unequally distributed; the hindleg in which the stronger rigidity developed, always showed the stronger F. R. Only in one preparation the F. R. at first was stronger in the leg in which the decerebrate rigidity was less pronounced, although also here after one hour the F. R. became more marked in the limb with the stronger decerebrate rigidity.

Fig. 1

Fig. 1 is part of a motion picture of a secondary decapitate cat in prone position on the operation table.

In pictures 1, 2, 3, 4, 5, 6 the right hindleg of the animal is extended by the observer. Between 6 and 7 it is released and snaps back into flexion.

The flexed position is reached in picture 10, showing that this flexion from the extended position occurs in about $\frac{1}{4}$ second.

Usually the knee reflexes can be easily obtained; sometimes, however, we had the impression that the contraction of the flexor muscles was so strong that it interfered with the eliciting of these reflexes. Occasionally, with moderate intensity of the F. R., weak homolateral flexion and contralateral extension reflexes can be elicited, superimposed upon this persistent flexion.

The consequences of the secondary decapitation on the front legs are less striking. In the great majority of cases decerebrate rigidity disappears and the fore limbs become flaccid.

In a few animals a slight amount of decerebrate rigidity persisted in the fore limbs after a decapitation, about 5 mm. caudal of the calamus. This has already been observed by Magnus (1926), who states that only after

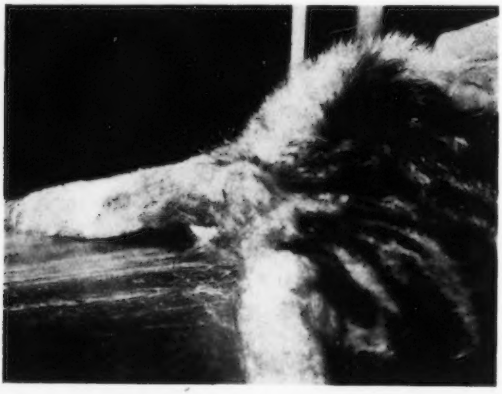

Fig. 2. Flexor rigidity and priapism in the "secondary" spinal cat (male).

decapitation about 11 mm. below the calamus decerebrate rigidity is completely abolished in the fore limbs in all cases.

Often, however, after a variable period ranging from one-fourth of an hour to a few hours, there appeared in the fore limbs a definite degree of contraction in the adductor muscles of the shoulder girdles and the flexors of the elbows.

Another feature of the picture which the secondary decapitate preparation presents is the appearance of marked *priapism* in the male cat. Often the erection of the penis is maximal, sometimes less pronounced (fig. 2). Dorsiflexion of the tail often elicits an augmentation of the erection, together with quick twitchings in the anal and perineal musculature as well as an increase of the flexion of the hind legs (see fig. 2). When priapism is well marked, dorsiflexion of the tail or tapping of the back of the animal

often provokes the ejection of a few small jets of urine or ejaculation of semen.

It is noteworthy that maximal priapism was observed in two distinct periods, namely, in most of the first 30 experiments during January and February, and in those performed after April 13. Priapism was much less pronounced and frequently absent in the experiments done during March and the first week of April. This is perhaps indicative of the presence of a seasonal factor in regard to this symptom; in this connection the statement in Brehm's *Tierleben* (1915) that the housecat gives birth to kittens during two periods, the first during April/May, and one during the beginning of August is of interest. Since the duration of pregnancy in the cat is 8 weeks, copulation would be most frequent during February and late spring.

Section of the posterior roots of the lumbosacral cord on one side immediately abolishes this flexor rigidity in the homolateral hind leg. Skinning of a hind leg results at first in a marked depression of the F. R. in that limb; after a few hours the rigidity may return to a certain extent, though we have not seen it attain its original value or the intensity of that in the contralateral hind leg.

It is significant that lifting the hindquarters of the preparation from the table by the tail, or placing the animal in the supine position results in a disappearance of the F. R. As soon as the animal is laid down again on the table, the F. R. reappears and the hindlimbs assume their typical position. Imitation of the stimuli which elicit the F. R. by rubbing the skin of the abdomen and of the inner aspect of the thighs and abduction of the legs, while the hindquarters of the animal are held lifted from the table, is followed by a definite reappearance of F. R. However, this F. R. is not as strong as that evoked by the contact with the table. Stretching the hindlegs when the animal is lifted from the table or is in the supine position does not elicit F. R.

Strong nociceptive stimuli applied to the erected penis, such as pinching this organ with an iris-forcep, often result in a temporary diminution or loss of the erection.

If the F. R. is only moderate in intensity, "tertiary" transection of the spinal cord at L. I. results in a marked augmentation of the F. R. In all cases in which this sequence of transections was followed, we have observed a maximal F. R. with strong dorsiflexion of the feet. As the participation of the ankle joints usually is least in the F. R. after secondary decapitation, the augmentation of the rigidity of their flexors is one of the most striking features after tertiary low spinal transection.

In a number of animals a low spinal transection at the level of L. I. was performed "secondarily." In these animals a marked or strong F. R. of the hindlegs also occurred. One of these showed the strongest F. R.

obtained in these experiments, as a tension of more than 2500 grams was required to extend the hindlegs.

In another series of 16 experiments (see table 2) we have investigated the significance of the time-interval between the primary decerebration and the secondary decapitation in the development of the syndrome described.

TABLE 2

Secondary decapitation after primary decerebration with various intervals between the two transections

NUMBER OF EXPERIMENT	INTERVAL BETWEEN TWO TRANSECTIONS	F. R.	ONSET OF F. R.	PRIAPISM	AUGMENTATION OF F. R. AFTER TERTIARY SPINAL TRANSECTION AT L. I.	REMARKS
53	14′	+++	Immediate	−	+	Castrated male
54	1 hr. 24′	−		−		
55	32′	++	Immediate	+	−	
57	40′	−		−	+	
58	10′	++	Immediate	++	+	
59	20′	+++	Immediate	−	+	
61	20′	++	Immediate	−	+?	
62	30′	++	1 hr. 10′		+	
63	30′			−	+	Persistence of D. R. in both hind legs abolished after transection at C IV. F. R. after transection at L. I.
64	50′	−		−	−	Persistence of D. R. in right hind leg. Both hind legs flaccid after transection at C III. No F. R. after transection at L. I.
66	17′	++	2 hr.	+	+	
67	37′	++	Immediate	+?	+	F. R. for at least 12 hrs.
69	40′	+	2 hr.	−	+	
70	50′	+++	Immediate	+	+	F. R. for 24 hrs.
71	1 hr.	+++	Immediate	+?	+	F. R. for at least 8 hrs.
72	1 hr. 15′	+++	?	−	+?	F. R. for at least 6 hrs.

From experiment 54, in which no F. R., nor priapism developed, it might seem as if in this respect a time factor was present. But experiments 71 and 72, in which a strong (+++), though not maximal (++++) F. R. set in, although there was an interval of 1 hour and 1 hour 15 minutes respectively between the decerebration and decapitation, show that this conclusion is not warranted. Perhaps prolonging the interval between these two transections results in a somewhat weaker F. R.

which occasionally sets in after a longer latency. The priapism was certainly much less pronounced than in experiments of table 1, but here the above mentioned seasonal factor may have entered, as these experiments were performed during the month of March. It would require special extensive investigation to settle this point.

The intensity of these phenomena may show slight fluctuations in the course of prolonged observation, but very often both the F. R. and priapism are very strong and even maximal over a period of many hours. This syndrome, flexor rigidity of the hindlegs associated with priapism, has been observed for as long as 26 hours. It seems as if such a secondary decapitate preparation is more resistant than the ordinary primary decapitate preparation, 24 hours being quite an unusually long time of survival for the latter.

In a few experiments secondary decapitation was performed in female cats. In these animals definite F. R. also appeared, but less marked than in the male cat.

Discussion. The flexor rigidity described in these experiments is a reflex phenomenon, since it is abolished by section of the posterior roots of the lumbosacral cord. Its diminution after skinning of a hindleg demonstrates the operation of exteroceptive stimuli, whereas its abolition after section of the posterior roots proves the concomitant activity of proprioceptive impulses in this reflex pattern.

The fact that passive extension of a hindleg does not produce F. R. when the animal is lifted from the table or is in the supine position, shows that this rigidity is not evoked by the passive stretching of the flexor muscles by the observer in testing the F. R.

Since this is the only manipulation of the preparation during the observations, the F. R. is apparently elicited by exteroceptive and proprioceptive stimuli arising from the contact of the lower part of the abdomen and the ventral aspect of the legs and the posture of these limbs when the animal is in the prone position on the table.

The abduction and strong flexion of the hindlegs is part of the normal copulation posture of the male cat. This abduction of the legs, together with the sensory stimulation of the skin of the abdomen and the medial aspect of the thighs, are probably the imitation in these experiments of the adequate stimuli which are active in the male cat during normal copulation by the contact of these regions with the hind quarters of the female.

It is plausible to interpret the adduction of the shoulders and the flexion of the elbows often present in these animals as part of a grasping reflex which may be seen in the frontlegs of the male during coitus. Furthermore, the augmentation of the F. R. and priapism together with ejaculation which results from dorsiflexion of the tail is in agreement with the fact that during copulation the tail of the male cat assumes a similar posture.

There can be little doubt, therefore, that this syndrome is the manifestation of a sexual reflex pattern, released in the lumbo-sacral cord after secondary spinal transection.

Since this syndrome does not occur after primary decapitation its appearance can not be explained on the basis of severance of anatomical pathways descending from the cerebrum or brain stem. *Therefore, a functional factor must operate.*

It has been shown by Wachholder that during strong decerebrate rigidity action currents are not only present in the extensor muscles, as is long known, but also in the flexors. This author concludes that the extension of the limbs in decerebrate rigidity does not prove an unequal distribution of central innervation, but is simply due to the greater strength of the extensor muscles.

This statement is not beyond criticism, since it may well be that the action currents appearing in the flexors during strong decerebrate rigidity are only strong effects of the marked action currents in the extensor muscles. However, if Wachholder's conclusion is correct, it is conceivable that secondary spinal transection, which abolishes the extensor factor in decerebrate rigidity, allows the flexor mechanisms to emerge and so results in F. R. We have tested this possibility in the following way.

In a few animals we eliminated in one hindleg the activity of the extensors, first of the hip, by freeing these muscles from their points of insertion, and then of the knee by sectioning the femoral nerve. Upon decerebrating this preparation the three intact limbs showed the usual decerebrate rigidity, whereas the operated hindleg remained flaccid. Subsequent decapitation produced typical F. R. in both hindlegs. Thus, if concomitant activity of flexor muscles exists at all in decebrate rigidity, it is not strong enough to cause flexion of a hindleg. Therefore, another functional factor must act.

This leads directly into a discussion of the still enigmatic problem of spinal shock. Sherrington, long ago, made the interesting observation that a second spinal transection performed after the "shock" following an initial low transection has disappeared does not result in a renewed depression of spinal activity.

The fundamental fact in our experiments is that marked flexor activity can be produced *immediately* after the secondary transection, showing absence of spinal shock as far as the flexor mechanisms are concerned.

Apparently by performing a secondary transection of the cord one eliminates, or greatly reduces, the time interval which exists for such a long time in the primary spinal preparation. Yet the question still remains why "shock" is absent or at least considerably less pronounced in the "secondary" spinal animal.

It is of interest that the blood pressure in such a preparation is just as

low as in a primary decapitate animal, showing that the spinal vasoconstrictor centres, in contrast to the flexor mechanisms, are in "shock" in the secondary decapitate preparation.

These experiments emphasize the general difference in distribution of extensor and flexor mechanisms in the neuraxis, although of course these two basic groups of reflexes are in the last analysis under the control of the entire central nervous system.

As is well known since the work of the schools of Goltz and Sherrington, some extensor activities are retained in the isolated spinal cord of the chronic preparation (homolateral extensor thrust and crossed extension reflex). Our experiments show that weak crossed extension reflexes can be obtained in the acute spinal animal against a background of moderate F. R. Such extensor activities, however, are meagre as compared with the mass of extensor reflexes of the brain-stem preparation (decerebrate rigidity and postural reflexes), indicating that undoubtedly the bulk of extensor mechanisms has shifted phylogenetically towards the brain-stem.

With regard to flexor reflexes it has long been known that they are the first to emerge in the later stages following spinal transection. The more recent observations mentioned in the introduction show that the flexor reflex may indeed be slightly augmented in the acute spinal preparation. These reactions represent primitive withdrawal responses to nociceptive stimuli mediated by the spinal cord. The experiments of this series strikingly demonstrate that under the special conditions of the acute secondary spinal preparation powerful, persistent flexor activity can be revealed which does not depend upon nociceptive stimuli, but is part of a sexual reflex pattern. Thus, important primitive flexor mechanisms, one for protection of the individual against noxious environmental influences, the other for the preservation of the species, are retained at spinal levels.

SUMMARY

1. In these experiments the results of a "secondary" spinal transection following "primary" decerebration of the animal (cat) are described.

2. In such an acute "secondary" spinal cat a syndrome appears which consists of strong springlike flexor rigidity (F. R.) in the hind legs associated with priapism.

3. This syndrome occurs in the secondary decapitate preparation as well as in the secondary low spinal preparation with transection of the spinal cord at L. I.

4. Section of the posterior roots on one side of the lumbosacral cord abolishes the F. R. in the homolateral hind leg, indicating that this F. R. is a reflex phenomenon.

5. Skinning of a hing leg after the establishment of F. R. results in a diminution of the F. R. Therefore exteroceptive impulses are involved.

6. From the observations under 4 and 5 it is apparent that proprioceptive impulses are also operating in the production of this F. R.

7. The F. R. disappears when the animal is lifted from the table or placed in the supine position showing that the adequate exteroceptive and proprioceptive stimuli initiating this reflex rigidity arise from the contact of the ventral aspect of the lower parts of the body with the table and the posture of the limbs in this prone position.

8. This syndrome is regarded as a copulation reflex pattern released in the lumbosacral cord after secondary spinal transection.

9. The absence or diminution of spinal shock as a possible explanation of this release is advanced.

10. The results of these experiments together with data from the literature indicate a general difference in the distribution of extensor and flexor mechanisms in the CNS.

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RESPONSE OF THE NICTITATING MEMBRANE TO PROLONGED STIMULATION OF THE CERVICAL SYMPATHETIC

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The present research was undertaken in order to study fatigue phenomena in the cervical sympathetic, and especially in order to see whether or not there are differences in the fatigability of the pre- and post-ganglionic elements.

A survey of the literature has shown that whereas the problems of fatigue have been fairly thoroughly studied in the cerebro-spinal system, very little, comparatively, has been done on that subject in the autonomic nervous system. Eve (1896), trying to find the histological changes produced by fatigue in the cell bodies in the superior cervical ganglion, excited during long periods the pre-ganglionic fibers of the cervical sympathetic and found that at the end of the longest stimulation (12 hours), the vaso-constrictor apparatus of a rabbit's ear was quite capable of vigorous action. He also found evidence of an acid reaction in the nerve cells of the superior cervical ganglion when directly stimulated or when stimulated through the cervical sympathetic under impaired circulatory conditions.

Brodie and Halliburton (1902) excited the splenic nerve and recorded the contraction of the spleen. They found that very rapidly a condition appeared similar to fatigue of the end organ in the nerve-muscle preparation. Blocking the impulses by cold, thus preventing them from reaching the end organ, they demonstrated that even after six hours of excitation, the nerve is practically as excitable as it was at the start, for a good splenic contraction is obtained when the cold block is removed. They also found that the post-ganglionic fibers, after six hours of excitation, were still able to maintain constriction of the arterioles, although the constriction showed a gradual decrease. There is no statement in either of the above mentioned papers about the frequency of stimulation, a condition of primary importance, as we shall see later.

METHODS AND RESULTS. The experiments were performed on cats. The cervical sympathetic nerve was carefully dissected and separated from the vagus, 2 cm. before and immediately beyond the superior cervical

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ganglion, between it and the skull. Two electrodes for excitation, conveniently isolated, were placed on the nerve 2 or 3 cm. before the ganglion and another pair of electrodes was hooked on the trunk beyond the ganglion. Since the post-ganglionic range was short, we cannot be sure that the stimulus did not spread to the ganglion itself. To prevent possible spreading of the current to other structures *via* the vagus the latter was severed just above the ganglion, before entering the skull, and also below, opposite the electrode on the pre-ganglionic fibers. Most of the experiments were performed on the right side.

The contraction of the nictitating membrane was recorded by means of a common muscle lever with a 4- or 5-fold magnification.

A Harvard inductorium, arranged so as to use the minimal stimulus to evoke a maximal effect, was employed to produce the excitation. A

Fig. 1. Contraction of the nictitating membrane. I, pre-ganglionic stimulation; II, post-ganglionic stimulation (42 break induction shocks per second). Time, 5 seconds. In this and the other figures the drop in the lowest line marks the period of stimulation.

test showed that it delivered about 42 break shocks per second. A double-throw double-pole switch allowed an almost instantaneous change of the place of excitation without varying the strength of the current.

As the investigation proceeded, the mode of excitation was altered. In early experiments the pre-ganglionic fibers were stimulated until the record showed a progressive decrease in the contraction of the membrane. As this was interpreted as a sign of fatigue, the excitation was quickly changed to the post-ganglionic fibers. Immediately the record showed a vigorous contraction of the membrane which persisted at the same level during excitation for much longer periods than the pre-ganglionic. Figure 1 is a typical record of this reaction, found in many animals.

In another series of experiments I determined the general form of the curve when the excitation was maintained for a long time, first on the pre-ganglionic fibers and afterwards on the post-ganglionic. The results were

quite constant. Figure 2 illustrates one of these experiments. The lower record displays the effect of continued excitation of pre-ganglionic fibers, the upper record that of post-ganglionic fibers. While the former clearly

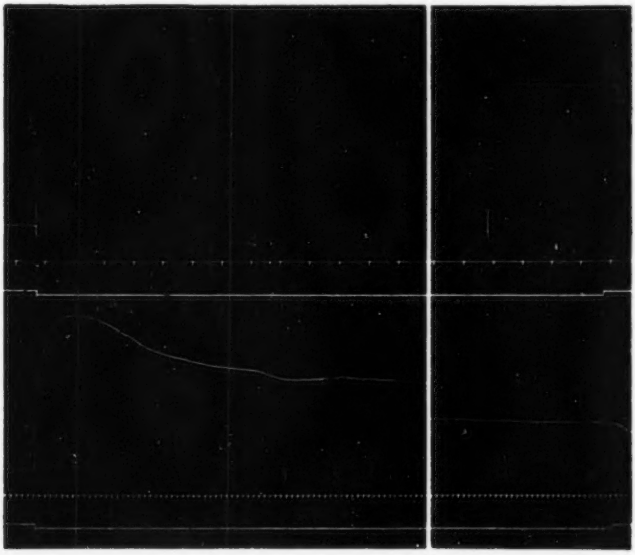

Fig. 2. Upper record: contraction of the nictitating membrane produced by prolonged post-ganglionic stimulation (42 break induction shocks per second). Time, 30 seconds. Lower record: contraction produced by pre-ganglionic stimulation (42 induction shocks per second). Time, 5 seconds. Between the two parts of the record there was an interval of 10 minutes.

Fig. 3. Record showing the importance of the blood supply in maintaining the contraction of the nictitating membrane produced by prolonged post-ganglionic excitation. Between the arrows both carotid arteries were compressed.

shows signs of fatigue, the latter shows none. The speed of the drum was approximately the same in both cases. The time in the lower tracing was recorded at intervals of 5 seconds and in the upper at intervals of 30 seconds. The total time of excitation was about 20 minutes.

In a third series of experiments I studied the influence of the blood supply on contraction of the membrane during excitation of post-ganglionic fibers. Figure 3 is a record of one of these experiments. During the period between the arrows the carotid arteries on both sides were completely blocked. As the record shows, the membrane immediately relaxed somewhat but with no tendency to reach the base line. When the blood was allowed to flow again, the membrane promptly contracted to the previous level. That this is not a passive, mechanical effect is proved by the fact that cutting off the blood supply when the membrane is not contracted produces no change.

Discussion. The peripheral neuromuscular mechanism involved in the contraction of the nictitating membrane includes the pre-ganglionic fibers, the post-ganglionic fibers, the connecting apparatus (ganglionic cells and synaptic junctions), and smooth muscle. Whenever the pre-ganglionic fibers are excited, the connecting apparatus is, of course, brought into play.

The experiments seem to show that while fatigue is rather readily produced by excitation of the pre-ganglionic fibers, it is much more difficult to provoke when post-ganglionic fibers are stimulated, even though the stimulation is prolonged. They also show the necessity of an adequate blood supply for the optimal function of the post-ganglionic system. These facts, however, should be carefully analyzed before drawing any conclusions.

Is it a real fatigue that happens as a consequence of the protracted pre-ganglionic excitation? The first possibility to consider is that the relaxation of the membrane in spite of the maintained stimulation is not a true fatigue, in the physiological meaning of the word, but the phenomenon analyzed by Howell, Budgett and Leonard (1894) as "stimulation fatigue," a term suggested by Gotch (1910) to indicate a loss of excitability at the point where the electrodes are applied. "Stimulation fatigue" was found to occur when sympathetic nerve trunks are excited during long intervals, especially when they are non-medullated, but without complete exclusion of the medullated fibers. If the electrodes are shifted peripherally along the nerve to a new place, the organ studied responds again with the same strength as before. Subsequently, Brodie and Halliburton (1902) confirmed these observations and showed also that when the nerve had lost its responsiveness at the points where the electrodes were located, it still was able to conduct impulses through that zone.

Apparently "stimulation fatigue" occurred to a certain extent in some of our experiments, because after a time shifting the electrodes along the

nerve in either direction produced a higher contraction; it did not, however, seem to be the predominant phenomenon. Indeed, the higher contractions thus evoked were neither equal in height to the first one nor did they last long. The two small increases shown in figure 1 during pre-ganglionic stimulation were caused by moving the electrodes to a new place.

In order to prove that "stimulation fatigue" is not an essential phenomenon and also in order to eliminate the more remote possibility of a functional fatigue along the nerve fibers, other experiments were performed in which the impulses were blocked before they reached the superior cervical ganglion, by means of a current of cold saline solution (4°C.), bathing the nerve at a convenient distance from the exciting electrodes. The nerve was constantly excited, and every now and then the block was removed by changing the cold flow to a warm flow of the saline solution. Whenever the block was thus removed, the membrane contracted to the same height as before or higher. In this case the excitation was maintained during 50 minutes; after that the experiment was discontinued because it seemed demonstrative enough. These experiments show clearly that the signs of fatigue observed when the pre-ganglionic fibers of the cervical sympathetic are excited are not due to a loss of excitability of the nerve either at the point stimulated or along the fibers.

Another possibility is that the relaxation of the membrane is due to a process of inhibition similar to that described by Wedensky (cited by Ioteyko, 1904) in the nerve-muscle preparation and depending on the frequency of stimulation. He found that for maintaining a muscle in tetanus at a steady tension, the frequency of the stimulation had to be decreased as the excitation was prolonged. In other words, when a muscle begins to relax in spite of a continued stimulation, it can be brought to the initial level by diminishing the frequency of stimulation, showing that inhibition rather than fatigue is the cause of the phenomenon. Similar observations to those of Wedensky have been more recently reported by Davis and Davis (1932).

In order to test the possibility of a phenomenon of this type, the experiments were repeated, exciting with the same inductorium but regulating the number of induction shocks by means of an adjustable rotary interrupter. The pre-ganglionic fibers were excited first with a frequency of about 42 break induction shocks per second as in our previous experiments, and when the nictitating membrane began to relax, the rate of stimulation was changed to 3 shocks per second. As demonstrated in figure 4, which is a typical record obtained under these circumstances, the membrane contracts again to an appreciable extent and stays contracted as long as the excitation persists. The same result occurs when the second stimulation is applied with frequencies ranging from 3 up to about 20 shocks per second.

After these experiments, which strongly support the view that the fatigue-like phenomena observed are probably of the inhibitory Wedensky type, we excited the pre-ganglionic fibers at a frequency of about 10 shocks per second during one hour, with no evidence of fatigue. Thereafter the nerve was obviously injured at the site of stimulation, probably because of the type of electrodes used, and no reliable information could be obtained. Prolonged excitation of post-ganglionic fibers with a similar rate produced no signs of fatigue for periods as long as an hour and 15 minutes.

From the evidence gathered in our experiments we can safely conclude that no signs of fatigue in the physiological sense of the word are evident in the contraction of the nictitating membrane after periods of one hour of constant stimulation of either pre- or post-ganglionic fibers *when adequate frequencies of excitation are employed.* If frequencies above a certain critical level are used, fatigue-like phenomena occur, undoubtedly related

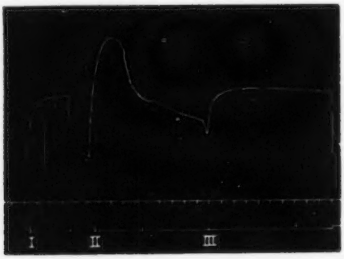

Fig. 4. Record illustrating the influence of the frequency of pre-ganglionic stimulation on the contraction of the nictitating membrane. I, stimulation at a rate of 3 induction shocks per second to test the effectiveness of the stimulus; II, prolonged stimulation at a rate of 42 shocks per second; III, 3 shocks per second.

to the Wedensky inhibition and depending upon the long refractory phase of the ganglion itself, which according to Bishop and Heinbecker (1932) is of the order of 20 to 30 sigma. This assumption is supported by the fact that the phenomenon does not appear during post-ganglionic excitation.

SUMMARY

The fatigability of the pre- and post-ganglionic neurones of the cervical sympathetic was investigated by prolonged stimulation of the pre- and post-ganglionic fibers under the same circumstances. The contraction of the nictitating membrane was used as an indicator.

When the pre-ganglionic fibers were stimulated continuously with an inductorium delivering 42 break induction shocks per second they soon failed to maintain the contraction of the membrane (see figs. 1 and 2).

When post-ganglionic fibers were stimulated under the same conditions, the membrane was maintained in the contracted state (see fig. 2).

That the relaxation observed during pre-ganglionic excitation is not true fatigue is shown by the fact that the membrane contracts again if the frequency of the excitation is decreased (see fig. 4). This pseudo-fatigue is probably related to the Wedensky inhibition; its presence only during pre-ganglionic excitation suggests that it is to be explained by the long refractory period of the superior cervical ganglion.

If pre-ganglionic fibers are stimulated with frequencies adjusted to the ability of the ganglion to transmit impulses, the stimulation may be continuous for at least an hour with no evidence of fatigue. Beyond that period, in our experiments, the observations were complicated by the local injury of the nerve fibers.

In conclusion, I wish to express my gratitude to Prof. Walter B. Cannon for his guidance and help and also for the facilities offered in his department.

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CAROTID SINUS REFLEXES TO THE RESPIRATORY CENTER[1]

I. Identification

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The recent discovery by H. E. Hering and his associates (1) that the carotid sinus is the source of afferent impulses capable of exerting important reflex effects has introduced a new factor into discussions of the regulation of the circulation. It has also led to the discovery that other vegetative functions, including respiration, are profoundly influenced by sinus reflexes. Recent disclosures indicate that the regulation of respiration is accomplished to a remarkable extent through sinus reflexes, and claims are made which, if fully valid, necessitate a fundamental revision in existing conceptions of the nature of respiratory control.

Sinus reflexes to the respiratory center were first investigated by Moissejeff (2) and subsequently by Koch (3) (4), but the most striking evidence and most drastic conclusions have been contributed by Heymans and his co-workers (5) (6) (7) (8).

In briefest résumé, this evidence is as follows: In intact animals (dogs) hyperpnea follows occlusion of the common carotid arteries; it is wholly reflex in origin because the effect is the same even though all branches of the carotids have been previously tied excepting only the lingual arteries, and because section of the sinus nerves completely abolishes the reaction (6). In dogs whose depressor nerves have been cut adrenalin produces apnea only if the sinus nerves are intact, so that adrenalin apnea is purely reflex (6). In experiments in which the sinuses of a dog are perfused with Locke's solution or shed blood, or supplied with blood from a donor animal, rise in endosinusal pressure causes respiratory depression or apnea, fall in pressure hyperpnea, both abolished by section of the sinus nerves (6). Changes in pH or CO_2 content of the Locke's solution perfused through the sinuses of dogs elicit marked respiratory effects; in crossed-circulation

[1] Preliminary reports of these experiments were presented before the Physiological Society of Philadelphia, May 18, 1931 (Amer. Journ. Med. Sci., 1931, clxxxii, 154) and the Federation of American Societies for Experimental Biology, April 28, 1932 (This Journal, 1932, ci, 91).

experiments inhalation of CO_2, nitrogen, or hydrogen by the donor dog leads to reflex hyperpnea in the recipient, and the reflex response may be greater than the direct effect of inhalation of the same gas by the recipient (7). In otherwise intact dogs denervation of the carotids greatly lessens or completely abolishes the respiratory responses to inhalation of CO_2, nitrogen, or hydrogen (7).

From these experimental results two conclusions of fundamental importance may be drawn: First, the respiratory center (in common with other vegetative centers) would be entirely unaffected by increase in its blood supply, any effect from rise in systemic or cephalic blood pressure being wholly due to reflexes from the sinuses and aorta. Second, the sensitivity of the cells of the respiratory center to changes in their chemical environment must be much less acute than had previously been supposed, the most highly specialized structures in this respect being the afferent end-organs of the carotid sinuses. The first conclusion invalidates Gesell's (9) conception of the regulation of respiration, the second necessitates a drastic revision of Haldane's (10). The respiratory center becomes a relatively insensitive and unimportant synapse in a reflex arc the physiologically specialized part of which is the end-organ in the wall of the internal carotid artery.

The experimental observations of Heymans and his collaborators have been partly confirmed by other workers. The presence of respiratory reflexes aroused by changes in endosinusal pressure is confirmed by Koch and Mark (4) and by Gollwitzer-Meier and Schulte (11) in dogs. The essentially reflex nature of adrenalin apnea is confirmed by Wright (12) in the decerebrate cat. Reflexes aroused by changes in chemical environment of the sinus end-organs, however, could not be demonstrated by Gollwitzer-Meier and Schulte (11) in experiments in which the sinuses of dogs were perfused with Locke's solution or beef blood of varied acidity or gas content. Cromer and Ivy (13) found that aseptic denervation of the sinuses did not appreciably modify the responses of unanesthetized dogs to controlled exercise—an observation which leads them to conclude that any important rôle of sinus reflexes in the regulation of respiration can readily be assumed by other mechanisms.

The experiments now to be described were intended to accomplish, first, a repetition of the observations of Heymans and his collaborators, and second, a subjection of the above conclusions to additional experiments intended further to test their validity. It may be said at once that ample confirmation of the existence and effectiveness of respiratory reflexes aroused by changes in endosinusal pressure has been secured; sensitivity of the sinus mechanism to reduction in oxygen tension of the blood has also been confirmed, but no comparable sensitivity to increase in CO_2 tension could be demonstrated; dependence of the respiratory re-

sponse to anoxemia upon sinus reflexes has also been confirmed, but that to CO_2 excess was found much less dependent upon reflexes (this paper). The results of other experiments, however, do not permit agreement with Heymans' contention that the respiratory (and vasomotor) center is insensitive to increase in its blood supply and that Gesell's conception of respiratory control is invalid (following paper).

I. Occlusion and release of the common carotid arteries. Ever since Hering proved that the hypertension which follows bilateral carotid occlusion is due to abolition of inhibitory tone exerted by the sinus reflex mechanism upon the vasomotor and cardio-regulatory centers (1), this procedure has been employed as a simple means for demonstrating the existence and intensity of that influence. Heymans (6) and Koch (4) have used it to demonstrate a similar tonic inhibitory influence upon the respiratory center. Heymans believes that the hyperpnea of carotid occlusion is not due to anemia of the center, as had previously been believed, but is entirely reflex in origin. His evidence is quite convincing: the hyperpnea occurs when all branches of the carotids have been tied excepting the linguals; it is completely abolished by sinus denervation; occlusion of the vertebral arteries has no such effect.

I have repeated these experiments on dogs and cats, using several different narcotics and decerebration in order to obtain an idea of the constancy and intensity of the effects. Eighteen experiments on dogs, with 44 carotid occlusions, and 10 on cats, with 37 occlusions, were deemed acceptable because blood pressure and respiration remained constant and reflexes were active. A number of additional experiments were discarded because of excessively deep narcosis or irregularity of blood pressure or respiration. The narcotics used were barbital, phenobarbital, chloralose, and morphine-urethane. Decerebration was done in 2 dogs and 5 cats by a ligature method (14); the external carotids were tied, but the internal carotids remained open throughout the actual experiment. Blood pressure was recorded from a femoral artery, using 25 per cent sodium thiosulfate or heparin-Ringer as the anticoagulant; a mercury manometer was used routinely. Respiration was recorded quantitatively, in cats and small dogs by the Lieb-Cushny (15) plethysmograph, in larger dogs by a Bohr meter connected to the expiratory side of a valved tracheal cannula with a pneumographic record of the rate. The carotids were occluded by rubber-covered bull-dog clamps, the edges of the wound being kept retracted by weighted hooks; every effort was made to avoid traction upon the vessels or irritation of surrounding tissues when the clamps were manipulated. The occlusions lasted one minute as a rule, preliminary experiments having shown maximal effects to be elicited within that time.

The results are illustrated in figures 1 and 2, which are constructed from the averages of all the observations made excepting those of two recent

experiments on dogs and three on cats. Inclusion of the latter does not materially alter the curves. The individual observations are also shown. Blood pressure was measured every five seconds and the curve is constructed

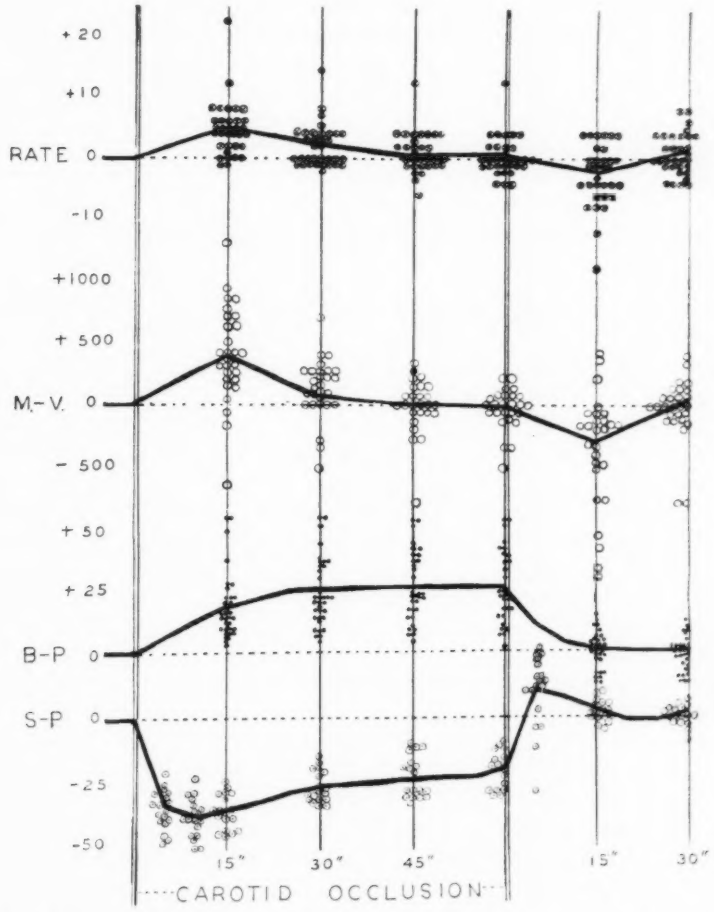

Fig. 1. Effects of carotid occlusion upon circulation and respiration of dogs. Results of 32 occlusions in 16 dogs, of which two were decerebrated; vagodepressor nerves intact in all cases. Curves represent averages of observations (shown as dots or circles) of changes in respiratory rate, *Rate*, respiratory minute-volume, *M-V*, femoral blood pressure, *B-P*, and endosinusal pressure, *S-P*, expressed as changes in respiratory rate or volume (in cubic centimeters) per minute, or in millimeters of pressure, with control value taken as zero.

from those measurements, but only the observations made at fifteen second intervals are shown. Respiration was measured every fifteen seconds.

Two effects were constant, namely, rise in blood pressure during the occlusion, and depression of both blood pressure and respiration upon release of the vessels. Respiratory stimulation upon occlusion was usual but not constant; occasionally respiration was only depressed. There was

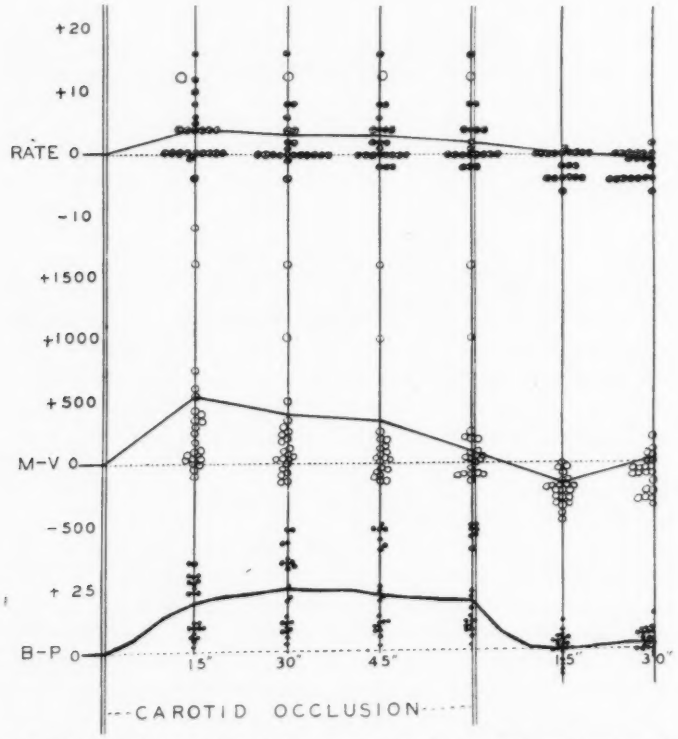

Fig. 2. Effects of carotid occlusion upon circulation and respiration of cats. Results of 33 occlusions in 7 cats, of which 6 were decerebrated; arrangement same as figure 1. Vagodepressor nerves intact.

no constant relation between the intensities of the circulatory and respiratory effects: a marked hypertension sometimes was associated with pure respiratory depression, or a marked hyperpnea with a relatively slight circulatory response. There was nothing to indicate that any of the narcotics used had had an appreciable influence upon the result; the responses were no more marked in decerebrate dogs and cats than in the narcotized animals.

These results are believed to be a fair representation of the regularity and intensity of the respiratory and circulatory effects to be expected from carotid occlusion in the narcotized or decerebrated dog or cat. It is noteworthy that while respiratory stimulation is usual it is not as constant as the circulatory response; more significant is the marked tendency of respiration to return toward normal after the first fifteen seconds of the occlusion, while the hypertension persists as long as the carotids are closed. These differences indicate that if the respiratory and circulatory effects are both wholly reflex in origin, there must be compensatory factors in the former, or else the two effects arise from separate sets of end-organs with different sensitivities to pressure and pulse changes.

Effect upon endosinsual pressure. In the interpretation of the above-described results, information was desired concerning the alterations in endosinusal pressure corresponding with occlusion and release of the carotids. To obtain it a cannula filled with heparin-Ringer solution was tied into the central stump of a lingual artery and connected to a mercury manometer. This was done in four dogs and one cat; in the latter repeated estimations were unreliable by reason of prompt clotting upon occlusion, but the first occlusion yielded good results (fig. 3) The results obtained during 18 occlusions in the four dogs are averaged in the curve labelled *S.P.* in figure 1; the individual observations are also shown. Measurements were made every five seconds, and these are shown for the intervals just after occlusion and release; elsewhere only the observations made at fifteen second intervals are shown, for the sake of simplicity. A typical result is shown in figure 3.

The actual pressures of this average curve were 107 mm. before occlusion, 69 at the lowest point (ten seconds after occlusion), 87 by the end of the minute of occlusion, and 118 five seconds after release.[2] Expressed as percentages, the abrupt fall in endosinusal pressure amounted to about 36 per cent of the control level of that pressure; the slow rise brought it to about 19 per cent below the control level; the abrupt rise just after release amounted to an increase of 36 per cent above the pressure existing just before release.

Correlating this curve with the others of figure 1 it is evident that the hyperpnea is produced only by a fall of some 35 per cent in endosinusal

[2] This fall is much less than that found by Heymans (5, p. 541) when he closed the carotid by which a sinus of one dog was joined to a donor animal: his figures show a fall from 150–170 mm. to 60–70 mm. in endosinusal pressure. The difference is probably due to the fact that in Heyman's experiment the outflow from the sinus entered directly into a jugular vein of the donor, so that peripheral resistance must have been abnormally low and collateral contributions to endosinusal pressure were excluded. In a preparation such as his it is impossible to deduce actual endosinusal pressure from the arterial pressure of the donor because of the low resistance on the outflow side.

pressure while the hypertension persists even when the pressure is less than 20 per cent below normal. This suggests that the two reflexes may arise from separate end-organs. An alternative possibility is that the hyperpnea is partly dependent upon anemia of the center, and that the hypertension removes this factor as it also raises endosinusal pressure. In order to decide between these possibilities, sinus denervation was resorted to.

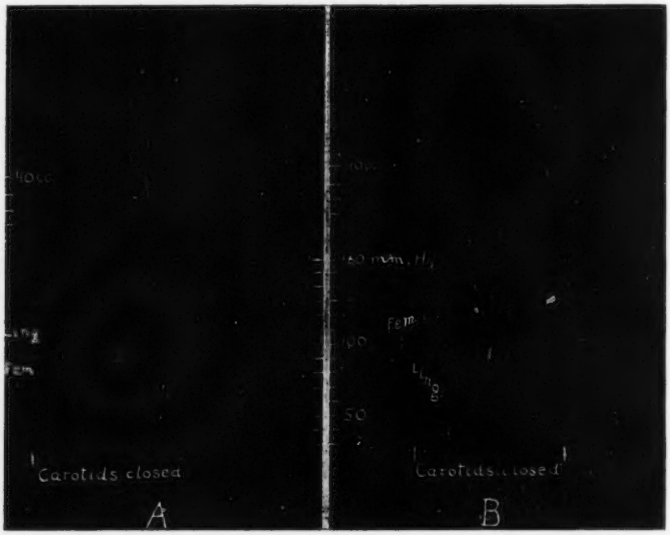

Fig. 3. Effect of carotid occlusion on respiration, blood pressure, and endosinusal pressure.

A. Dog—barbital. Depressor nerves cut (Koch's method), vagi intact. Respiratory plethysmograph. Sinus (lingual) and systemic (femoral) pressures recorded by mercury manometers, zero for both at time tracing.

B. Cat—phenobarbital; vagi and depressors intact. Arrangement same as in A.

Effect of sinus denervation This was done in nearly every one of the experiments but only the results obtained in five dogs and three cats were regarded as valid because in the others there was hypertension or irregular breathing after the denervation. The degree of cerebral anemia produced by carotid occlusion could scarcely be as great in the presence of hypertension as it was before, and irregular breathing indicated a change in the animal's condition that probably was not entirely due to removal of sinus reflexes. The denervation was accomplished in all cases by complete excision, between ligatures, of all attachments of the sinus regions. In each case a series of occlusions was made both before and after the denervation.

The results are well illustrated by the outcome of two representative experiments on dogs, as shown in figure 4. The circulatory response was regularly and (except for a negligible mechanical effect) completely abolished. The respiratory effect was generally reduced and frequently abolished,

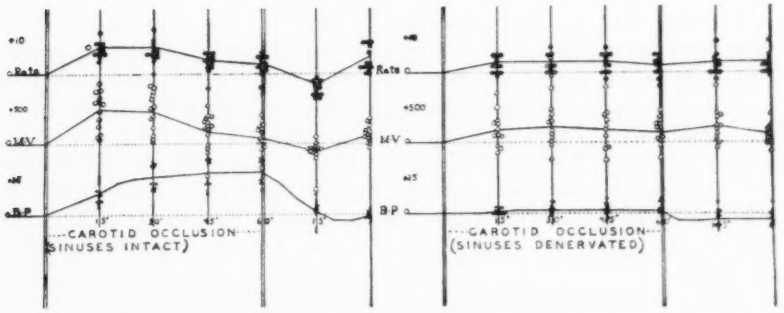

Fig. 4. Influence of sinus denervation upon respiratory and circulatory responses to carotid occlusion. From two experiments on barbitalized dogs. Arrangement same as figure 1.

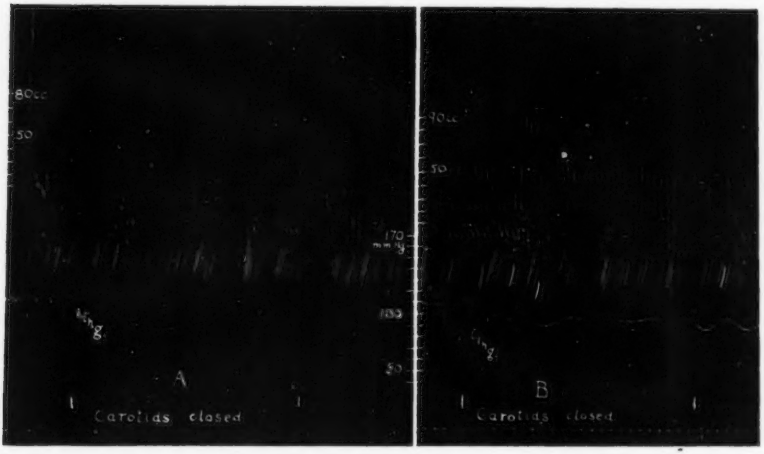

Fig. 5. Influence of sinus denervation on respiratory and circulatory responses to carotid occlusion. Dog—phenobarbital; vagus and depressor nerves intact. Respiratory plethysmograph, femoral and lingual pressures, as in figure 3.

A—sinus nerves intact; occlusion 2 minutes. Respiratory minute-volumes: 2183 cc. before, 2574 cc. during, and 2039 cc. after occlusion.

B—both sinus regions excised; occlusion 2 minutes. Respiratory minute-volumes: 2005 cc. before, 2924 cc. during, 2224 cc. after occlusion.

but in nearly every experiment there was at least one instance of definite hyperpnea from occlusion of the denervated vessels. Exceptionally the effect was even greater after the denervation than before (fig. 5). It must be emphasized that hyperpnea was much more capricious in its occurrence after the denervation, and that instances of its complete absence were much more frequent. This makes the result very difficult to interpret, for if hyperpnea upon occlusion of the denervated vessels is due to central anemia it should be repeatable. On the other hand, if it were due entirely to sinus reflexes it should never occur after the denervation. The only justifiable conclusion is that the hyperpnea of carotid occlusion is largely but not wholly, usually but not always, due to sinus reflexes, and that factors which have not yet been identified operate to make uncertain the results of occlusion of the denervated vessels.

TABLE 1

Carotid and vertebral occlusion—effects upon cerebral blood flow

	REDUCTION IN CEREBRAL VENOUS OUTFLOW ON CLAMPING		
	Carotids	Vertebrals	Both
	per cent	*per cent*	*per cent*
Dogs	55	33	86
	40		66
	26	13	70
	48	0	
	54	25	83
Average—Dogs	45	25	76
Cat	55		86

Effect upon cerebral blood flow. Heymans and Bouckaert (6), in support of their contention that the effects of carotid occlusion are wholly reflex in origin, cite experiments in which they find that occlusion of the vertebral arteries has no comparable effects although medullary blood flow should be reduced at least as much by the latter as by the former. I have repeated this experiment on cats and dogs and have confirmed their observation: occlusion of both vertebrals does not cause hypertension or hyperpnea. Their interpretation, however, is not obligatory because they present no evidence that vertebral occlusion actually does reduce central blood flow as much as carotid occlusion does. Before accepting their conclusion it seemed advisable to test by actual experiment the relative effectiveness of carotid and vertebral occlusion in reducing cerebral blood flow.

The method used was that of measurement of venous outflow from the

torcular Herophili, as described in an earlier publication (16). Five dogs were used. The results are summarized in table 1. The percentile reductions represent the average results of three or more occlusions. The results of one earlier experiment on a cat, using the superior caval method (16), are also included. The narcotic was barbital in all cases.

It is not claimed that this method is a precise one, but the results were sufficiently consistent to warrant the conclusion that the degree of cerebral anemia produced by carotid occlusion in the dog is considerably greater than that which follows vertebral occlusion. Absence of hyperpnea from vertebral occlusion may therefore merely mean that the anemia so produced was not intense enough.

SUMMARY

The results of these experiments, while generally confirmatory of Heymans' conclusions, disclose differences between the respiratory and circulatory effects which indicate that the situation may not be as simple as he believed. The evanescence of the hyperpnea, its lack of correspondence with the circulatory effect, and its occasional occurrence after sinus denervation, all indicate that the respiratory effects of carotid occlusion are at least partly or occasionally dependent upon something other than inactivation of the entire sinus reflex mechanism. It is conceded that sinus reflexes are usually responsible for much of the hyperpnea, often for all of it, but the capriciousness of the respiratory effect after sinus denervation points to the intervention of factors which have not yet been identified.

II. Respiratory reflexes from controlled changes in endosinusal pressure-perfusion experiments. The experiments now to be described represent efforts at repetition of those reported by Heymans and his co-workers (6) (7). Cats and rabbits were used as well as dogs. Both carotids were exposed and, with the aid of a loupe, all vascular branches were tied excepting the external carotids or linguals, in which the efferent cannulae were inserted. The Richards-Drinker (17) pump was used in most experiments; in seven dogs the technic of vessel-to-vessel anastomosis, as employed by Heymans, was utilized.

The procedures used in most of the experiments are shown in diagram in figure 6. This method of crossed-perfusion had the following advantages: first, the blood used for perfusion was taken from and returned to the circulation of a living donor, so that its chemical composition could either be kept constant or altered within limits known to be physiological; second, perfusion pressure being determined by the hydrostatic resistance on the outflow side, it could be set at any desired level or varied within any predetermined limits, depending only upon the position of the mercury reservoir, and regardless of changes in blood pressure in the donor; third, for the same reason the rate and type of pulsation within the sinuses was the

same at all levels of perfusion pressure above zero, so that the influence of changes in pressure could be separated from that of changes in pulsation; fourth, it was possible to test for patent carotid branches in the recipient by the addition of adrenalin to the perfusing blood while maintaining perfusion pressure at a much higher level than systemic: patency even of a tiny vessel was manifested as a rise in systemic pressure. Coagulation of the donor's blood was prevented by intravenous injection of heparin (0.1 gram per kilo). The methods of recording respiration and blood pressure were those described in the preceding section (p. 96).

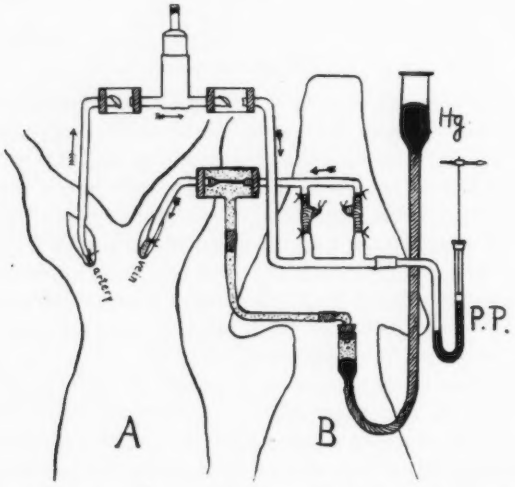

Fig. 6. Crossed-perfusion of carotid sinuses. Blood drawn from a femoral artery of donor (A) is driven by pump through both common carotids of recipient, (B), through cannulae in external carotids or linguals of B, through a thin-walled rubber tube surrounded by saline in a glass chamber, and returned to a femoral vein of donor. External pressure upon rubber section of outflow tube is regulated by adjusting height of mercury leveling-bulb (Hg). Endosinusal pressure is recorded by mercury manometer (P.P.).

The validity of each preparation depended upon reactivity of the sinus mechanism and upon absence of vascular communications between donor and recipient. The first was disclosed by the circulatory effects of alterations in endosinusal pressure, the second by the adrenalin test alluded to above. If the latter indicated a patent vessel, the experiment was not continued until closure had been effected. The additional control of noting the effect of sinus denervation was also used frequently, but only in cases in which it was possible to cut the sinus nerves without mass ligation. Otherwise this is not an adequate check on the dissection because

ligation must of necessity occlude patent vessels, so that the denervation may mean interruption of vascular as well as nervous communication between donor and recipient. The narcotics used were barbital, phenobarbital, amytal, chloralose, and morphine-urethane. A number of experiments were also made upon decerebrate dogs and cats. Of the experiments attempted by this method, 23 on dogs, 11 on cats, and 5 on rabbits were valid in that changes in endosinusal pressure had definite reflex effects and there were no vascular communications between donor and recipient.

The number of experiments was large because in most of them a study was made of "chemical" as well as "pressure" reflexes, and while in the cats it was possible in every case to demonstrate sensitivity to both types of stimuli, it was not possible to obtain "chemical" reflexes in dogs until 18 experiments had been made with this method and 6 by direct anastomosis. Reasons for this will be considered in the following section of this paper.

The results were a complete confirmation of those obtained by Heymans and Bouckaert (6), Koch and Mark (4), and Gollwitzer-Meier and Schulte (11) in that rise in endosinusal pressure caused respiratory depression or apnea with as great uniformity as could be expected in experiments involving as much abnormality as these. Fall in endosinusal pressure likewise caused respiratory stimulation in many cases, but this effect was decidedly less constant than the opposite one of rise in pressure. In further confirmation of Heymans (6), the respiratory (and circulatory) effects of changes in endosinusal pressure were regularly and often very markedly enhanced by section of the vago-depressor or depressor nerves, and were completely abolished by denervation of the sinuses. The results are best illustrated by selected examples from the original records (figs. 7, 8, 9, 10). These are unusually marked effects, but it seems justifiable to regard them as the closest approach to the effects that might be elicited in the absence of deleterious influences such as narcosis, dissection, and artificial circulation. I have also been able to confirm most of Heymans' observations upon dogs with direct vessel-to-vessel anastomosis.

In addition to this confirmation of the results of other investigators, the use of a somewhat different method and of animals that had not previously been used in studies of sinus respiratory reflexes made it possible to secure additional data of some interest. They are as follows:

First, the intensity of the respiratory response to alterations in endosinusal pressure bore no constant relation to that of the circulatory. This is particularly noticeable in comparing the results obtained with cats with those of the rabbit experiments. In the cat the respiratory effects were relatively marked, the circulatory relatively slight, while in the rabbit the circulatory effects were great, the respiratory slight, often completely absent (figs. 9 C and 10). In dogs both types of relation were encountered, but the most striking respiratory effects ocurred in animals whose circulatory re-

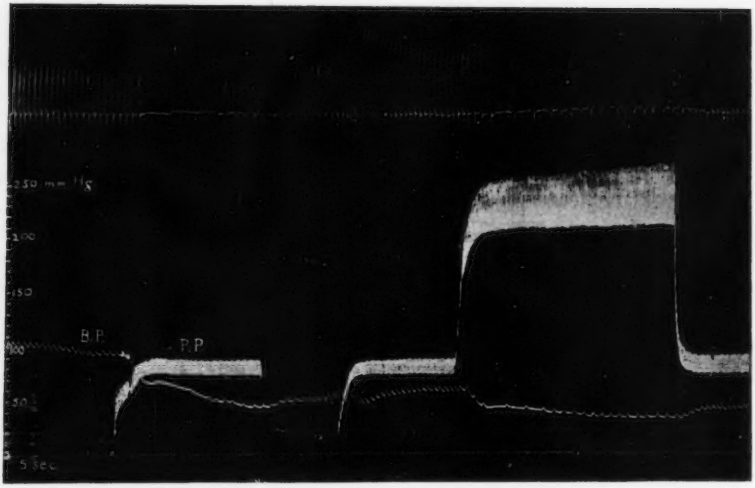

Fig. 7. Effects of alterations in endosinusal pressure—dog. Crossed-perfusion; amytal in donor, barbital in recipient. Vagi of recipient cut, respiration by pneumograph. Perfusion and systemic pressures of recipient by mercury manometers, zero at time tracing.

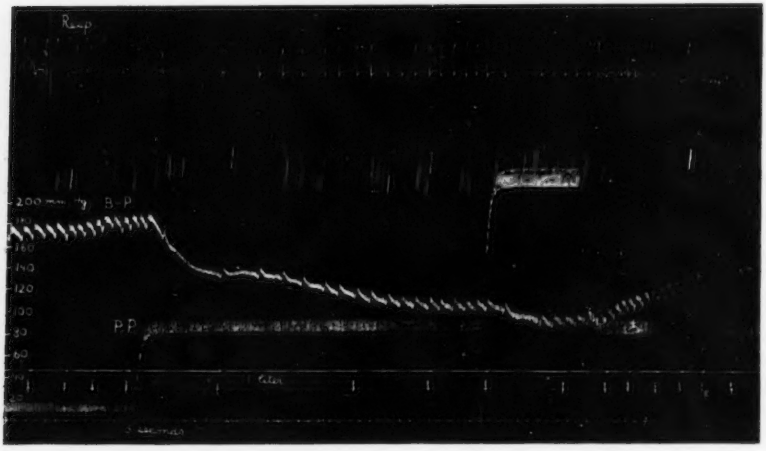

Fig. 8. Crossed perfusion of carotid sinuses—dog. Barbital in donor and recipient. Vagi of recipient cut; mercury manometers, zero at time tracing. Respiration of recipient by pneumograph and by measurement of expired air: each mark of signal (line just above time tracing) represents one liter measured by Bohr meter.

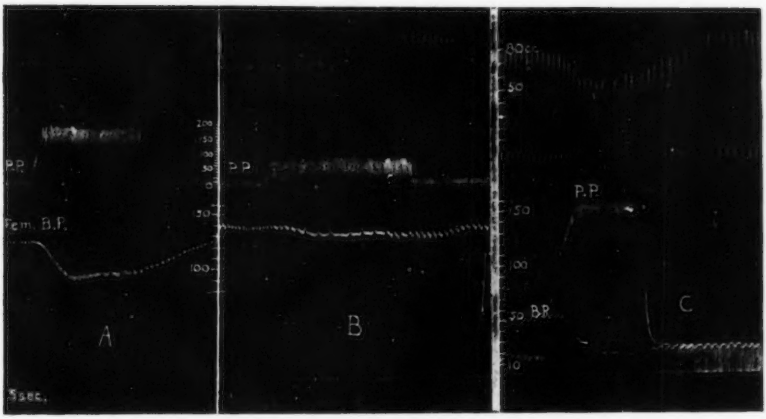

Fig. 9. Perfusion of carotid sinuses—dog and rabbit

A and B from same experiment on barbitalized dog, vagi cut. Sinuses perfused with Locke's solution. Perfusion pressure by membrane manometer, systemic by mercury manometer; respiration by pneumograph.

C—rabbit: barbital. Depressor and vagus nerves intact. Sinuses perfused with heparinized rabbit blood. Perfusion and systemic (subclavian) pressures by mercury manometers, zero at time tracing. Respiration by plethysmograph.

Fig. 10. Crossed-perfusion of carotid sinuses of cat. Barbital in donor and recipient; vagi intact. Mercury manometers, zero at time tracing; respiratory plethysmograph.

sponses were relatively slight; in those dogs in which the circulatory effects were most marked the respiratory effects were often comparatively slight.

Second, in all the animals there was evident a striking tendency of respiration to escape from the first effect of alteration in endosinusal pressure when the pressure was maintained at a new level. Blood pressure showed much less tendency to escape, and often showed none at all (figs. 7 and 8).

Third, the respiratory response was essentially a response to change in endosinual pressure, not to any absolute level of pressure, while the circulatory response was more nearly proportional to the actual pressure level. Thus, a first rise in endosinusal pressure commonly caused apnea even though the actual pressure was lower than arterial, but an additional rise to a high level had less effect, and reduction to the level at which the initial apnea had occurred now caused hyperpnea (figs. 7, 8, and 10). The circulatory effects were more nearly proportional to the actual level of endosinusal pressure.

Fourth, the respiratory effects were greater when the perfusion pressure change began or ended at zero than they were over any other range, even though the actual changes in pressure were much greater in the latter cases. The circulatory effects were more nearly proportional to the actual change in pressure. In decerebrate cats the effect of stopping the pump was commonly to cause intense dyspnea, which, in one case, culminated in violent generalized convulsions; on restarting the pump there was an apnea. These marked effects were elicited by changes in endosinusal pressure amounting to as little as 15 mm. Hg, and were not accompanied by corresponding changes in systemic blood pressure (fig. 11). Similar observations were also made in dogs, though less frequently (fig. 7). It would seem that the respiratory reflexes are more affected by abolition and restoration of pulsation than by actual endosinusal pressure, since the effects were greatest when the pump was stopped and restarted.

Finally, there were instances—most frequent in cats—of distinct respiratory reflexes without any change in blood pressure, and of respiratory stimulation coincident with a marked hypotension following rise in endosinusal pressure (fig. 11).

These observations indicate that the respiratory reflexes aroused by changes in endosinusal pressure do not represent simply the irradiation of excitations intended primarily for the vasomotor center, as Koch (3) believes. It seems most probable that they arise from end-organs that are separate and distinct from those concerned in the circulatory reflexes. Additional reason for this belief is the influence of "chemical reflexes" upon circulation and respiration. These will be considered in the following section of this paper, but the evidence bearing upon the point now under consideration may properly be adduced here. In the decerebrate cat re-

breathing caused intense reflex hyperpnea without any significant change in systemic blood pressure (fig. 13). In the dog anoxemia caused reflex hypotension, while CO_2 excess caused reflex hypertension; both caused reflex hyperpnea (figs. 14 and 15).

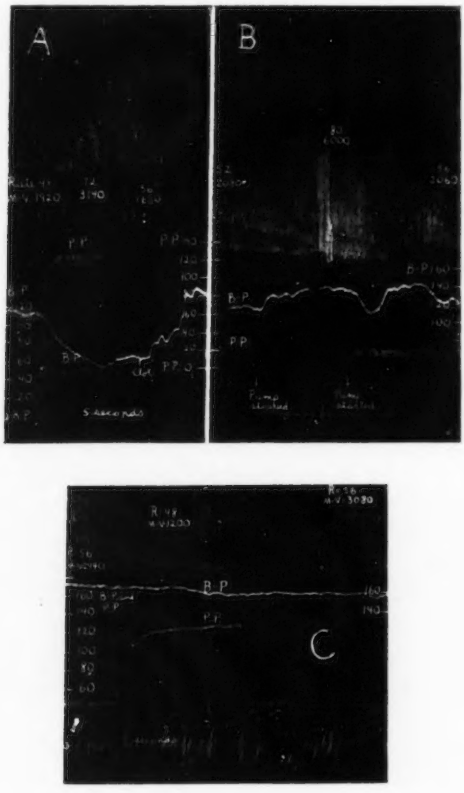

Fig. 11. Crossed-perfusion of carotid sinuses of decerebrate cats

A and B from same experiment: barbital in donor, vagi of recipient intact; mercury manometers, set at different zero levels (calibrations shown); respiratory plethysmograph.

C—another experiment, arrangement same as in A and B; vagi intact.

III. Respiratory reflexes from changes in chemical composition of blood within the carotid sinuses. The experiments now to be described represent an attempt at repetition of those of Heymans, Bouckaert, and Dautrebande (7) from which the conclusion was derived that sinus

reflexes play a prominent part in the chemical regulation of respiration. They were of two general sorts: first, determination of the extent to which the respiratory response to inhalation of CO_2 or nitrogen was modified by acute denervation of the sinuses; second, crossed-perfusion and crossed-circulation experiments in which the donor animal was made to breathe

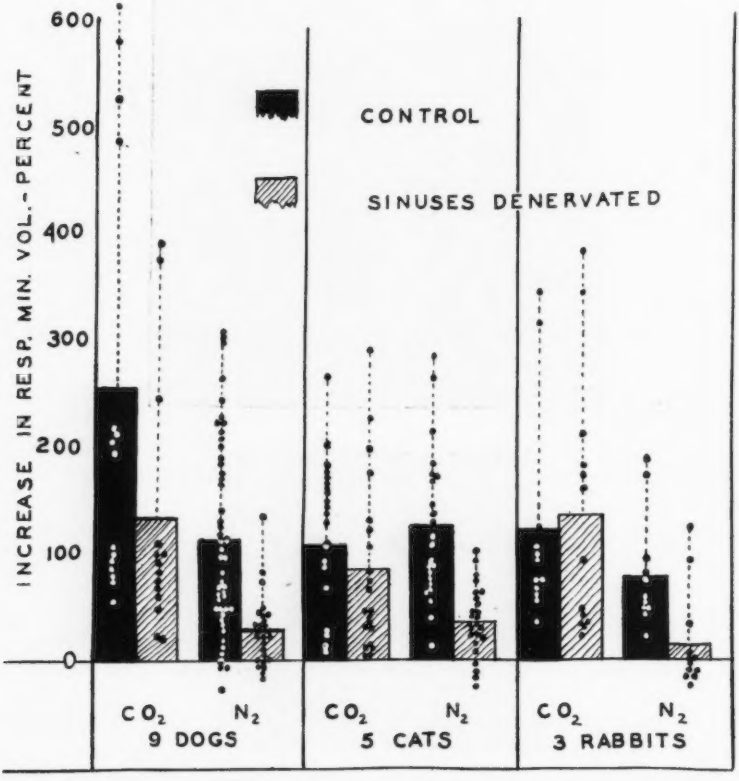

Fig. 12. Influence of sinus denervation upon respiratory response to inhalation of CO_2 and nitrogen. Blocks represent averages of observations which are shown as dots. Vagi intact in all cases.

appropriate gas mixtures. The outcome, while somewhat less striking than that of the similar experiments made by the Belgian workers, nevertheless represents confirmation of their general conclusions.

1. *Influence of sinus denervation upon the respiratory response to inhalation of CO_2 and nitrogen.* The experiments were made upon 9 dogs, 5 cats,

and 3 rabbits. The animals were routinely narcotized with soluble barbital (0.25 gram per kilo intraperitoneally). Registration of blood pressure and of volume of breathing was done as usual (p. 96); the anticoagulant in the arterial cannula was thiosulfate or heparin-Ringer solution. The gases used were pure nitrogen and CO_2 (10 per cent) in oxygen. They were inhaled for one minute as a rule; in some cases the nitrogen inhalation had to be shortened to 20 or 30 seconds because of cardiac effects. A rubber

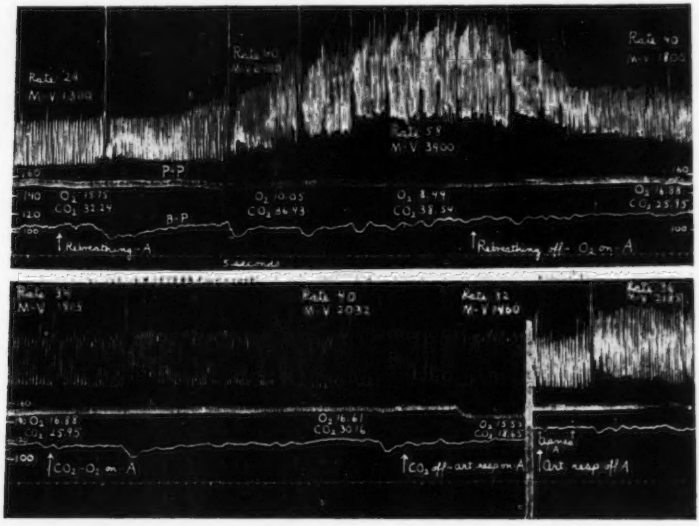

Fig. 13. "Chemical reflexes" from carotid sinuses of decerebrate cat. Crossed perfusion, barbital in donor; vagi intact. Mercury manometers set at same zero level—calibration shown; respiratory plethysmograph. Rate and minute volumes, *M-V*, of respiration of recipient, oxygen and CO_2 content of donor's blood, shown on tracing.

A—rebreathing by donor.

B—CO_2-O_2 (10 per cent–90 per cent) inhalation by donor.

C—removal of artificial respiration from donor, followed by brief apnea. Follows directly after B.

bag attached directly to the tracheal cannula was used in cats and rabbits, a Douglas bag attached to the inspiratory valve in dogs. In cats and dogs the influence of section of the aortic (vagodepressor) nerves was tested, but in the rabbits only the sinus nerves were cut. After a series of inhalations (usually 5) of each gas, the sinuses were denervated by complete division, between ligatures, of all attachments; in the rabbits the internal carotids were spared because they play a relatively great part in the cere-

bral blood supply of this animal, but in cats and dogs they were cut. After the denervation another series of inhalations of each gas was given.

The results are summarized in figure 12, in which the individual observations as well as their averages are shown in terms of percentile increase

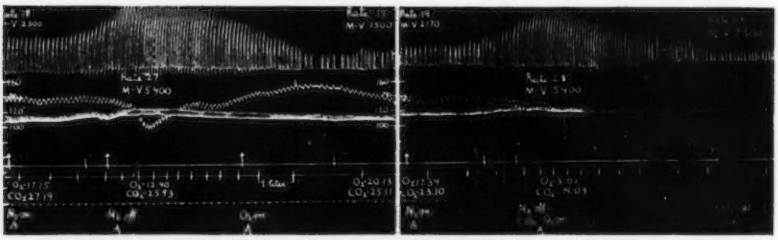

Fig. 14. "Chemical reflexes" from anoxemia in dog. Crossed-perfusion: barbital in donor and recipient; vagi of recipient cut; mercury manometers set at zero on time tracing; respiration of recipient by pneumograph and measurement of expired air (signal line above time tracing shows each liter expired). Rate and minute-volume of recipient's breathing and oxygen and CO_2 content of donor's blood shown on tracing.

A and B—nitrogen inhalation by donor. In A, oxygen, in B, CO_2—10 per cent in oxygen, given donor by tracheal catheter. Unfortunately no blood sample was obtained in latter case.

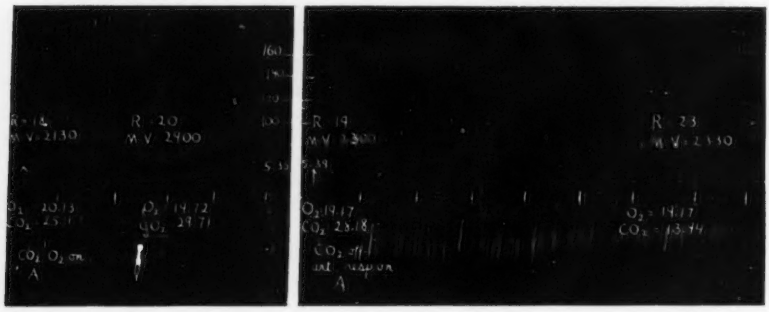

Fig. 15. "Chemical reflexes"—maximal effects of changes in CO_2 content of blood. Same experiment as figure 14. Note relatively slight and brief respiratory effect of CO_2 inhalation (10 per cent in oxygen), with definite hypertension, latter removed by reduction in blood CO_2 content by artificial ventilation; B taken 4 minutes after A, CO_2 inhalation continuing until beginning of B.

in minute-volume of breathing. They clearly confirm the results of the similar experiments of Heymans, Bouckaert, and Dautrebande (7) as far as nitrogen inhalation is concerned: this was almost always made less

stimulant by the denervation, and quite frequently it became purely depressant. The same was true of the circulatory effects also. The response to inhalation of CO_2 in oxygen was not markedly reduced by the denervation in any of the animals, and in the rabbits it was not reduced at all. It may therefore be concluded that in the barbitalized dog, cat, and rabbit the stimulant effects of anoxemia (nitrogen inhalation) are always largely, sometimes wholly dependent upon sinus reflexes, while those of CO_2 excess are mainly central.

The effect of section of the aortic (vagodepressor) nerves in dogs and cats was usually to reduce to the vanishing point the stimulant response to anoxemia remaining after sinus denervation. In cats and dogs there was often some stimulant effect as long as the aortic nerves were intact, but this was not true in rabbits, in which anoxemia was usually purely depressant after sinus denervation alone. From this it appears that aortic reflexes likewise play a considerable part in the anoxemic response of the dog and cat, not in that of the rabbit. In no case did aortic and sinus denervation remove the stimulant effect of CO_2 inhalation.

2. *"Chemical reflexes" in crossed-circulation experiments.* These experiments were intended to demonstrate the presence or absence of a physiologically important sensitivity of the sinus end-organs to changes in chemical composition of the blood. The method of crossed-perfusion of the sinuses (fig. 6) was used as well as Heymans' technic of direct anastomosis by means of Payr tubes. The latter was employed in seven successful experiments on dogs; it was tried in cats, but clotting prevented useful results. Crossed-perfusion was preferred because it could be used in cats as well as dogs; because endosinusal pressure could be maintained constant in the face of chemical changes in the donor's blood; and because it permitted a conclusive test for the absence of patent carotid branches in the recipient (by means of adrenalin) before the actual experiment began. Respiration and femoral blood pressure of the recipient were recorded as usual. Blood samples, collected from the pump intake, were analyzed for their oxygen and CO_2 content by the manometric method of Van Slyke and Neill (18).

Changes in chemical composition of the carotid blood were induced by subjecting the donor to rebreathing, to artificial respiration, and to inhalation of CO_2 (10 per cent in oxygen), nitrogen, and oxygen. The changes were known to be within physiological limits because they were induced in a living donor. No attempt was made to repeat Heymans' experiments (7) in which saline fluid or shed blood of varied pH or gas content was used for perfusion because the chemical changes used by him were beyond the physiological range. The point of major interest was not the ability of end-organs to respond to a change in their environment, but the physiological significance of such response.

The results were as follows:

In cats results similar to those reported by Heymans et al. (7) were obtained consistently from the start. Attempts at determining the intensity which this effect could attain led to the employment of decerebrate recipients. The most marked effects observed are shown in figure 13. It should be noted that in this animal, in which rebreathing by the donor caused a reflex hyperpnea amounting to a 200 per cent increase in minute volume, changes in CO_2 had only trifling effects; that this difference was not due to loss of reactivity by the animal is shown by the definite reflex hyperpnea caused by the suspension of the donor's breathing upon cessation of artificial respiration (fig. 13) after the CO_2 experiment had been made.

In dogs repeated attempts to obtain similar results were unsuccessful although all of the animals were responsive to changes in endosinusal pressure and some of them were exceedingly so. Heymans (7) (8) having expressed the belief that the "chemical reflexes" probably arise from end-organs separate from those responsible for "pressure reflexes," as suggested by deCastro, the preparation was modified in various ways without success. Finally it was decided to apply to the dog all details of the preparation used in the cat in which positive results had been obtained regularly. The only change consisted in ligation of the occipital arteries at a distance from the parent vessel, using the line of the exposed sinus nerve for reference, whereas in the preceding experiments the occipital artery had been tied close to the carotid for the sake of convenience (fig. 16). As soon as this change was made positive results were obtained consistently in dogs: 5 crossed-perfusions and one direct vessel-to-vessel anastomosis have been made, and the results confirm those of Heymans.

The most striking of these effects are shown in figures 14 and 15. These, as well as the other results with dogs and cats, show anoxemia to be decidedly more effective than CO_2 excess in arousing sinus reflexes. In fact it was possible in this experiment to stop the reflex hyperpnea of anoxemia by substituting 10 per cent CO_2 in oxygen for the nitrogen previously inhaled by the donor (fig. 14).

These results in both dogs and cats conform with those of the denervation experiments in indicating that the sinus mechanism is concerned much more with anoxemia than with CO_2 excess. The effects were proved to be due entirely to reflexes, not only by the routine demonstration of absence of vascular communications between donor and recipient, but also by the effect of section of the sinus nerves without ligation. In the crossed-perfusion experiments endosinusal pressure remained constant, so that the reflex respiratory effects were not contaminated by pressure reflexes.

The outcome of the final experiments on dogs not only furnished confirmation of Heymans' results; it also confirmed his belief that the

mechanism involved in the response to chemical changes is probably distinct from that concerned with pressure changes. Among the 18 experiments in which the occipital arteries of dogs were tied close to the carotids were some of the preparations that were most sensitive to changes in endosinusal pressure (figs. 7 and 9); in none of these were there any definite "chemical reflexes." In the six experiments in which the occipital arteries of dogs were tied at a distance of 5 to 10 mm. from the carotids, there were definite and fully reversible "chemical reflexes" in all, but in only one of them was there outstanding sensitivity to changes in endosinusal pressure; in one there were practically no respiratory responses to changes in pressure, yet there were marked reflex effects from anoxemia in the donor. For these reasons it seemed probable that the receptors involved in "chemical reflexes" are situated in the first portion of the occipital artery in the dog

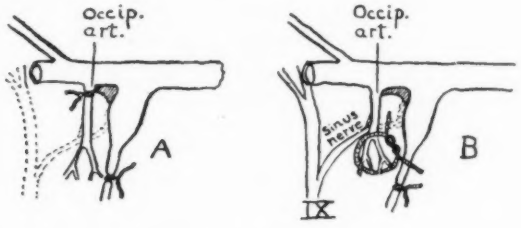

Fig. 16. Change in dissection which furnished positive "chemical reflexes" in dogs.

A—occipital artery tied at origin from carotid: pressure reflexes present, chemical reflexes absent.

B—occipital artery tied beyond line of exposed sinus nerve: pressure and chemical reflexes both present.

and not in the carotid sinus itself. An attempt was made to demonstrate this by a simpler technic, as follows:

Using a vagotomized dog, "pressure reflexes" were elicited by occlusion of the common carotids, "chemical reflexes" by inhalation of nitrogen. A series of occlusions and inhalations was made as a control, then both occipital arteries were tied close to the carotids (fig. 16A) and another series of observations was made. Finally both sinus nerves were cut and a third series of observations was made. Four experiments were performed, barbital being the narcotic.

The results are summarized in figure 17. The chemical response (nitrogen inhalation) was reduced from an average increase of 115 per cent to one of 54 per cent by occipital ligation, while sinus denervation reduced it further to one of 22 per cent: occipital ligation therefore accounted for 66 per cent of the total reduction. The pressure response was changed

by occipital ligation from an average increase of 33 per cent to one of 30 per cent, while sinus denervation reduced it to one of 3 per cent: occipital ligation therefore accounted for only 10 per cent of the total reduction—a change which is not regarded as significant.

These results are presented as evidence that in the dog most of the end-organs which are responsible for the respiratory reflexes aroused by chemical changes in the blood are situated in the first portion of the occipital artery; these reflexes can be greatly weakened or entirely prevented by ligating the vessel at its origin (presumably because this prevents access of the altered blood to the receptors) without interfering appreciably with the respiratory reflex mechanism involved in pressure changes.[3]

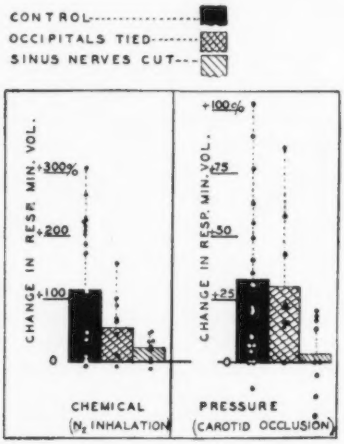

Fig. 17. Influence of occipital ligation upon respiratory responses to chemical and pressure changes—dogs. Blocks represent averages of observations, which are shown as dots. Occipital ligation at level shown in figure 16A.

CONCLUSIONS

1. The respiratory effects of occlusion of the common carotid arteries of dogs and cats, while generally comparable with the circulatory effects,

[3] This conclusion should be modified in accordance with recent experiments made in Heymans' laboratory (Annal. de Physiol. et de Physico-Chim. Biol., 1931, vii, 207), to which Dr. Heymans has just called my attention. Evidence is there presented that the chemically sensitive receptors are situated in the carotid body which, Dr. Heymans informs me, receives its blood-supply from the occipital artery. My results indicate only that these receptors are supplied with blood from this artery; they do not permit definite localization.

are less constant and less persistent than the circulatory. They are usually but not always purely reflex in origin because sinus denervation does not always abolish them, although it makes them much more capricious in their occurrence. Endosinusal pressure is reduced by about 36 per cent at first, but it rises during the occlusion until it is less than 20 per cent below normal; hyperpnea disappears as endosinusal pressure rises, but hypertension persists, so that both effects can scarcely be due entirely to inactivation of the same sinus reflex mechanism. Carotid occlusion reduces cerebral blood flow by about 45 per cent in dogs, while vertebral occlusion reduces it only by about 25 per cent: the greater effect of the former upon respiration may therefore be partly due to its greater influence upon blood supply of the center.

2. In perfusion experiments upon dogs, cats, and rabbits, rise in endosinusal pressure caused respiratory depression or apnea, while fall in pressure caused hyperpnea; the former effect was more constant and usually more striking than the latter. Section of the depressor nerves enhanced these effects and section of the sinus nerves abolished them.

3. The respiratory effects elicited by changes in endosinusal pressure probably arise from structures that are distinct from those concerned in circulatory reflexes because the intensities of the two effects are often entirely independent; because the respiratory effects are less persistent than the circulatory and less proportional to the actual level of endosinusal pressure than to the level from which the pressure was changed; and because occasionally there may be definite reflex respiratory effects without any circulatory response whatever. The respiratory mechanism appears to be relatively more sensitive to changes in pulsation than to changes in mean pressure, although it is influenced by both.

4. Participation of sinus reflexes in the chemical regulation of breathing is indicated by marked reduction or abolition of the response to anoxemia as a result of sinus denervation and by crossed hyperpnea in a recipient animal when asphyxia or anoxemia is induced in the donor whose blood is used to perfuse the sinuses. In both types of experiment the effects of CO_2 appear to be much more central than reflex.

5. The mechanisms involved in chemical reflexes are probably distinct from those concerned in the responses to pressure changes. In the dog they appear to be located mainly in the first part of the occipital artery.

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CAROTID SINUS REFLEXES TO THE RESPIRATORY CENTER[1]

II. Attempt at Evaluation

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The experiments described in the preceding paper (1) fully confirm the finding of Heymans and Bouckaert (2) that the respiratory center may be profoundly influenced by reflexes aroused in the carotid sinuses by changes in endosinusal pressure. The physiological significance of this reflex mechanism to the control of respiration remains to be determined. According to Heymans (3) (4), the vasomotor and cardio-inhibitory centers are completely unaffected by any physiological changes in flow or pressure in the cephalic circulation; although acute anemia or high external (intracranial) pressure may have direct effects upon them, the physiological responses of these cell-groups to changes in cephalic blood pressure he believes to be entirely due to sinus reflexes. The experiments now to be reported were intended to determine whether this conclusion is applicable to the respiratory center. Direct stimulant effects of acute anemia being conceded, attention was directed mainly to the production of increase in medullary blood flow under such circumstances that sinus (and aortic) reflexes could not contribute to the result. Four different means were employed for the purpose; with two of them a period of acute anemia preceded the increase in blood flow, but with the other two the blood supply of the center was adequate for normal breathing before the flow was increased. The common result of all four sets of experiments was respiratory depression or apnea that could not have been due to known reflexes, that was not always attributable to preliminary exposure to acute anemia, and that is believed to be an indication that the respiratory center is by no means insensitive to alterations in its blood supply, even within physiological limits. In a few other experiments similar observations were made upon the vasomotor center.

1. The effect of release of occluded carotid and vertebral ar-

[1] An exhibit of the results of some of these experiments was given at the meeting of the American Medical Association, June 8–12, 1931 (Journ. Amer. Med. Assoc., 1931, xcvi, 1599).

TERIES. When the common carotid and vertebral arteries are closed simultaneously violent dyspnea results, and if they are reopened while the dyspnea is at its height apnea occurs. These effects have long been known (see 5). The apnea might be due to abrupt increase in blood supply of the brain, or to sudden reinstatement of inhibitory sinus reflexes, or to both influences combined. In order to estimate the importance of sinus reflexes, the carotids were occluded alternately centrally (common carotid) and peripherally (external and internal carotids) to the sinuses, and before and after section of the sinus nerves. The results in four experiments on

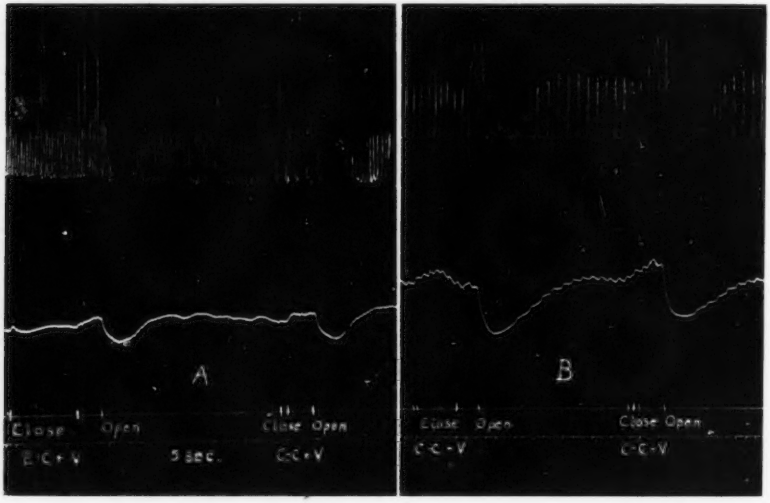

Fig. 1. Apnea upon release of occluded carotid and vertebral arteries—cat. Barbital; respiratory plethysmograph; femoral blood pressure—zero at time tracing.

A—vagus and sinus nerves intact; internal carotids tied throughout. First occlusion and release—external carotids and vertebrals; second, common carotids and vertebrals.

B—vagi cut, sinus regions excised. Occlusion and release of carotids and vertebrals now produce longer apnea than before.

dogs and three on cats were so uniform and unequivocal that additional observations seemed unnecessary. Characteristic results are illustrated in figures 1 and 2.

In no case was there any sign that carotid sinus reflexes played any part in the apnea produced by release of the occluded vessels. The apnea was just as long when endosinusal pressure rose during occlusion and fell upon release (external and internal carotid occlusion) as it was when the pressure was abruptly raised from a subnormal level (by reopening the

common carotids). Denervation of the sinuses had no influence on the result. To show that the apnea was not due to loss of CO_2 from the blood during the hyperpnea resulting from the occlusion, the experiment was repeated during inhalation of 10 per cent CO_2. The apnea produced by release of the vessels was not prevented thereby (fig. 2).

This experiment was also tried upon three rabbits narcotized with urethane (two) or ether. In no case was there any hyperpnea upon occlusion or apnea upon release of the carotids and vertebrals, the only effect upon breathing being pure depression by the occlusion and return to normal

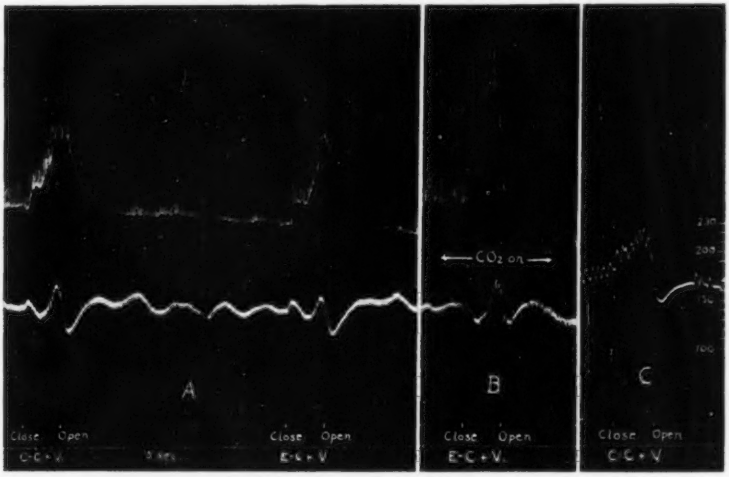

Fig. 2. Apnea upon release of occluded carotid and vertebral arteries—dog. Barbital; respiratory plethysmograph in A and B, pneumograph in C; otherwise same as figure 1.

A—vagi and sinuses intact, air breathing.

B—CO_2–O_2 (10 per cent–90 per cent) inhalation throughout—otherwise same as A.

C—vagi cut, sinus regions excised.

upon release. The effects upon blood pressure were of the same nature as those observed in dogs and cats but they were more marked in the rabbits. The reason for this failure of the respiratory center of the rabbit to respond like that of the dog and cat has not been investigated further.

2. THE EFFECT OF SUDDEN REDUCTION IN HIGH CEREBROSPINAL PRESSURE. This procedure was employed as a means for producing a marked increase in cerebral blood flow without the intervention of consensual changes in aortic or carotid pressure. Dogs were used. Cerebrospinal pressure was raised by means of a suspended reservoir of Locke's solution connected

(through a warming coil and a T-tube, to permit instantaneous reduction in pressure) to a metal cannula screwed into a trephined opening in a parietal bone, or to a needle introduced into the cisterna magna. Five experiments were made, with uniform results. Examples are shown in figures 3 and 4.

When cerebrospinal pressure was suddenly raised to a level close to or higher than that of arterial blood pressure, blood pressure rose and respi-

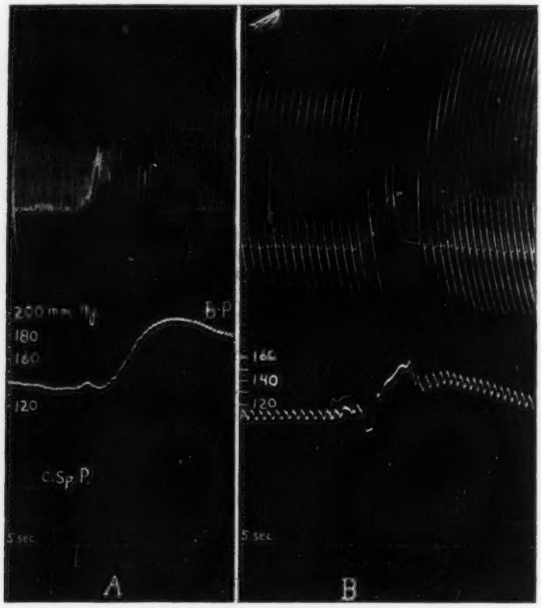

Fig. 3. Apnea upon sudden reduction in high cerebrospinal pressure—dog. Barbital; respiratory plethysmograph; cisternal needle connected to mercury manometer to record extramedullary pressure—this and femoral blood pressure both based upon time tracing.

A—aortic nerves cut (Koch's method), both sinus regions excised leaving internal carotids open. Rise and fall of cisternal pressure are shown in rectangular excursion of manometer lever.

B—vagi also cut; note inspiratory spasm without hyperpnea upon elevation, and apnea upon reduction of cerebrospinal pressure.

ration was stimulated. When cerebrospinal pressure was suddenly lowered during this stage there was an immediate apnea. Section of the sinus and vago-depressor nerves did not modify the result. Inhalation of CO_2 throughout the period of elevation and lowering of cerebrospinal pressure diminished but did not prevent the apnea produced by the lowering.

The effects of elevation of cerebrospinal pressure are generally believed to be due to reduction in blood flow through the finer cerebral vessels (6), (16). Sudden reduction of high intracranial pressure may therefore be assumed to act by increasing cerebral blood flow. In any case, neither the hyperpnea of elevated intracranial pressure nor the apnea of sudden reduction of the pressure is dependent upon sinus or aortic reflexes, as shown by the lack of influence from section of the sinus and aortic nerves.

3. EFFECTS OF ADRENALIN AND OF DEPRESSOR DRUGS AFTER SECTION OF SINUS AND AORTIC NERVES. Heymans and Bouckaert (2) were unable to

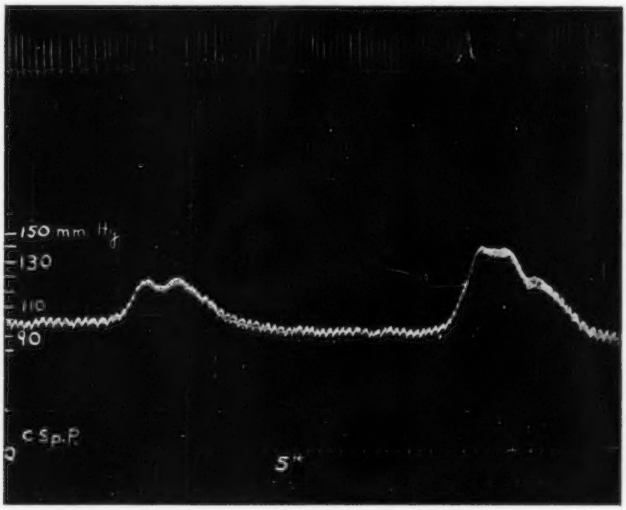

Fig. 4. Apnea upon reduction of high cerebrospinal pressure—dog. Barbital; pneumograph; cisternal needle; sinus regions excised, leaving internal carotids open; aortic and vagus nerves intact. Otherwise same as figure 3.

produce apnea by intravenous injection of adrenalin in dogs after section of the sinus and aortic nerves. A similar result was obtained by Wright (7) in decerebrate cats. They conclude that adrenalin apnea is wholly due to reflexes from the sinuses and aorta.

This is not supported by the results of these experiments. It is true that in nearly every case in which adrenalin was injected before and after the denervation apnea was no longer produced by it on the latter circumstances. But if the denervation was done at the outset, the first dose of adrenalin commonly caused an apnea. Furthermore, in the crossed-perfusion experiments described in the preceding paper (1) adrenalin was frequently in-

jected intravenously in animals whose carotid systems were isolated and whose vago-depressor nerves were cut. Apnea was a common result, and it was sometimes more marked when carotid pressure was low than when it was high. Nitroglycerine and acetyl choline, injected intravenously in dosage sufficient to lower systemic blood pressure, were also found to be capable in some animals of causing hyperpnea although the sinus and aortic nerves were cut.

Examples of these results are shown in figures 5, 6, and 7. These justify the conclusion that adrenalin apnea is not wholly reflex in origin.

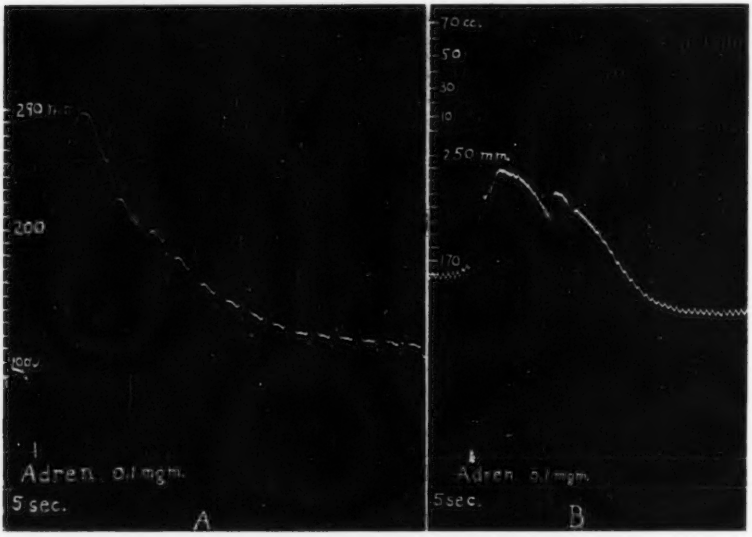

Fig. 5. Adrenalin apnea in decerebrate animals with aortic and sinus nerves cut.

A—dog; pneumograph; vagi cut; sinus regions completely excised, including internal carotids; external carotids tied; femoral blood pressure.

B—cat; respiratory plethysmograph; vagi cut; sinus regions excised, including internal carotids; external carotids tied.

The failure of adrenalin to produce apnea after sinus denervation can frequently be duplicated if repeated injections are made with some other operation between them, leaving the sinus nerves intact. The reason for this is not part of the present problem.

Brief mention may also be made of some experiments which indicate that the vasomotor center, like the respiratory, is depressed by adrenalin through mechanisms other than sinus and aortic reflexes. One kidney of a dog was perfused in situ by means of a pump with blood taken from and

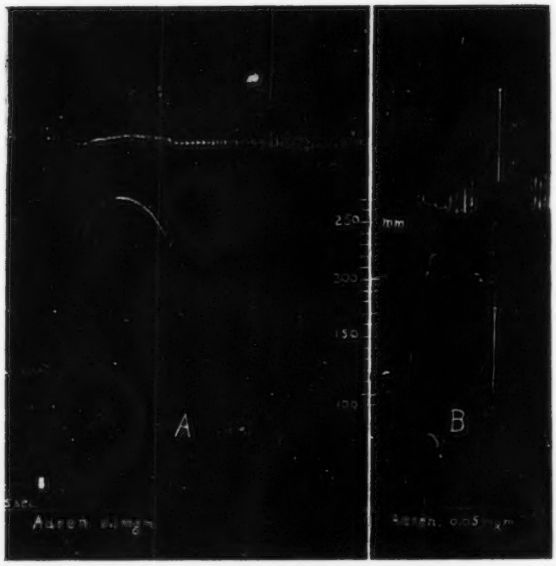

Fig. 6. Adrenalin apnea in animals with aortic nerves cut and sinus pressure under perfusion control.

A—dog—barbital; vagi cut; pneumograph; crossed-perfusion of both sinuses—pump stopped, endosinusal pressure zero.

B—cat—barbital; vagi cut; respiratory plethysmograph; crossed-perfusion of sinuses at constant pressure throughout.

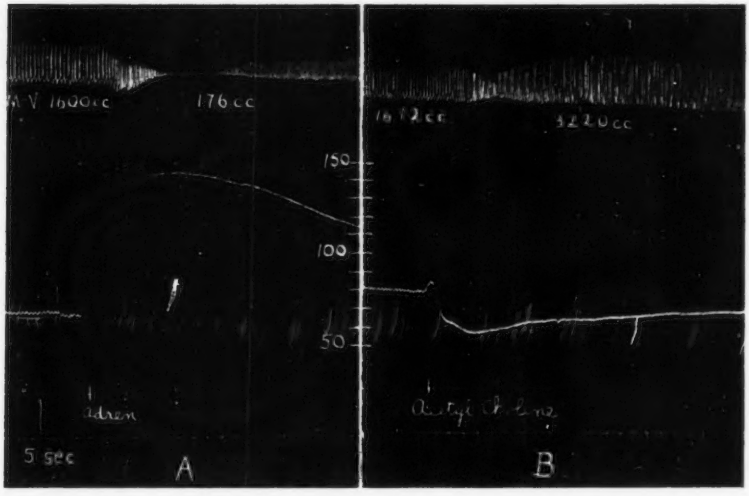

Fig. 7. Respiratory effects of adrenalin and acetyl choline in rabbit with depressor and sinus nerves cut. Barbital; depressors cut, sinus regions excised, leaving internal carotids open; vagi and sympathetics intact; subclavian blood pressure.

A—adrenalin—0.05 mgm. by jugular vein.

B—ten minutes after A; acetyl choline (0.005 mgm. by jugular vein).

returned to the circulation of a donor animal. The vasomotor connections of the kidney were intact, as shown by marked rise in perfusion pressure despite constancy of pump output when asphyxia was induced in the recipient by occlusion of the trachea. Two experiments were made. In both the vago-depressor nerves were cut and carotid sinus reflexes were excluded, in the first by clamping both common carotids, in the second by complete section of all attachments of the sinus region. In one artificial respiration was maintained throughout. Adrenalin, injected intravenously in the experimental animal, caused a fall in perfusion pressure as systemic pressure rose (fig. 8). Since rate of perfusion flow did not diminish, this can only mean decrease in tone of the renal vessels mediated through their

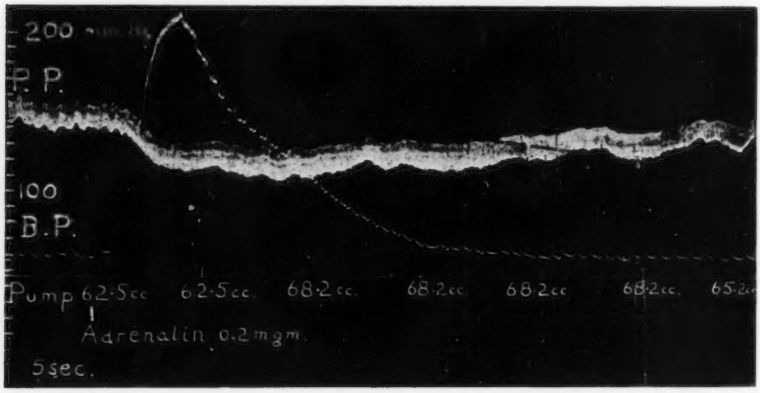

Fig. 8. Depression of vasomotor center by adrenalin hypertension in dog with aortic and sinus nerves cut. Crossed-perfusion of kidney in situ; barbital in donor and recipient; vagi cut and sinus regions excised in recipient; donor under constant artificial respiration throughout; adrenalin injected into femoral vein of recipient. Note fall in kidney perfusion pressure (*P.P.*) without reduction in pump output. Respiration of recipient was not depressed by the injection. Pump output (*Pump*) shown in cubic centimeters per minute.

nerve supply, and the indicated vasomotor depression could not have been due to reflexes from the sinuses or aorta.

From the above experiments it is concluded that the respiratory and vasomotor centers are depressed by the rise in blood pressure produced by adrenalin through some agency other than sinus and aortic reflexes.

4. RESPIRATORY EFFECTS OF CONTROLLED CHANGES IN CEREBRAL BLOOD FLOW WITH SINUS AND AORTIC REFLEXES EXCLUDED (PERFUSION OF THE CEREBRAL CIRCULATION). In an earlier publication (5) a report was made of the respiratory responses of cats and dogs to alteration in the rate of flow of blood perfused through the vertebral arteries: respiration, in

favorable experiments, was consistently stimulated by reduction and depressed by increase in vertebral blood flow. Those results are not discredited by the recent disclosures of the importance of sinus reflexes because the common carotids were clamped in every case, and sinus reflexes are presumably inactivated thereby. Aortic reflexes likewise could have played no favorable part in the results because the nature of the experiments demanded a greater withdrawal of blood from the systemic arterial circulation when vertebral flow was increased, so that aortic pressure was necessarily decreased whenever vertebral flow was increased. The instances

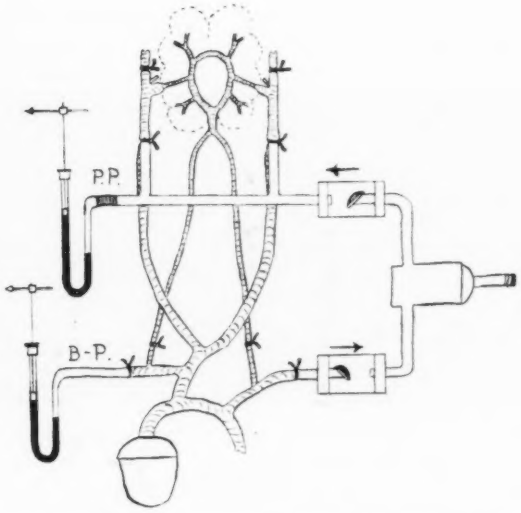

Fig. 9. Method for perfusion of brain of rabbit with animal's own blood. Outflow of pump enters cannulae tied into common carotids; external carotids and vertebral are tied; sinus regions are excised, leaving internal carotids open. Pressures recorded by mercury manometers.

cited in the paper referred to (5, fig. 9) are therefore valid evidence that the respiratory center is directly influenced by changes in its blood supply in either direction.

It seemed advisable, however, to make absolutely certain that aortic and sinus reflexes could be excluded without abolishing the respiratory responses to changes in cerebral perfusion flow. For this purpose a series of experiments was made in which the brain was perfused by way of the internal carotids, before and after section of the sinus nerves, and with the aortic nerves divided. Rabbits were used because their internal carotids are relatively large, because their intracranial circulations are not in free

communication with extracranial areas, and because section of the aortic nerves is readily accomplished without division of the vagus trunks.

The method is shown in diagram in figure 9. The narcotic was soluble barbital (0.25 gram per kilo intraperitoneally). The carotid and subclavian arterial systems were dissected free, all branches of the latter being tied excepting the vertebrals which were prepared for subsequent occlusion by means of loose ligatures. The external carotids and all carotid branches other than the internal carotids were tied. Blood was taken from one subclavian artery to supply the Richards-Drinker pump, by which it was driven through the cephalic ends of the common carotids. A by-pass to a jugular vein was provided to prevent perfusion of the brain with saline or with stagnant blood at the outset. Clotting was prevented by intra-

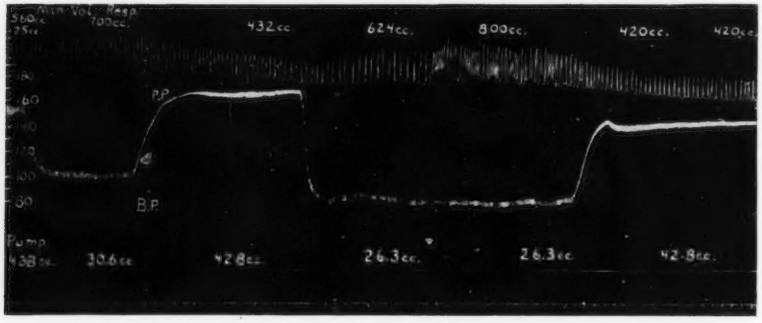

Fig. 10. Respiratory effects of alterations in internal carotid perfusion flow in rabbit. Barbital; respiratory plethysmograph; depressor and sinus nerves cut; perfusion pressure, "*P.P.*" and subclavian blood pressure, "*B-P.*" based upon time tracing. Respiratory minute-volumes and cerebral blood flow, *pump* are shown as cubic centimeters per minute.

venous injection of heparin (0.1 gram per kilo). Respiration was recorded plethysmographically and the aortic nerves were cut at the start of each experiment.

The results of the four successful experiments of this nature are summarized in table 1, and some of the most striking effects are illustrated in figure 10. In the first of these experiments the animal's circulation failed after the sinuses were denervated, but the results up to that time are included because they illustrate the sort of effect to be expected when sinus reflexes are operative. In the last one the sinuses were denervated at the start, while in the other two definite results were obtained both before and after the denervation. Table 1 also shows a summary of the results of the most favorable of the earlier vertebral perfusion experiments.

These results are presented as the final piece of evidence that the respiratory center is directly affected by changes in its blood supply.

TABLE 1

Respiratory effects of changes in cerebral perfusion flow

Barbital narcosis in all cases. Figures for cerebral blood-flow and respiratory minute volume (M-V) represent cubic centimeters per minute; respiratory rate per minute also shown (Rate). Depressor nerves cut in rabbits, intact in dogs and cats.

	PERFUSION VIA	INCREASE IN CEREBRAL BLOOD-FLOW								DECREASE IN CEREBRAL BLOOD-FLOW								SINUS NERVES
				Effect on respiration				Per cent change in				Effect on respiration				Per cent change in		
				From		To						From		To				
		From	To	Rate	M-V	Rate	M-V	Flow	Resp. M-V	From	To	Rate	M-V	Rate	M-V	Flow	Resp. M-V	
Rabbits	Int. carotids	16.3	28	72	432	56	168	+72	−61	22.9	16.3	72	432	72	720	−29	+66	Intact
		16.6	48.4	68	340	60	120	+191	−65	48.4	16.4	60	120	60	480	−195	+300	Intact
		3.6	20.4	52	312	76	228	+466	−26	11.2	3.6	76	304	52	310	−211	+2	Cut
		17	29.7	64	2,560	64	2,240	+70	−11	23.1	17	72	2,520	64	2,560	−26	+2	Intact
		25.6	39	40	1,280	32	640	+52	−50	42.8	26.3	24	432	40	800	−38	+85	Cut
		18.7	22.4	40	1,080	32	800	+20	−26	34.9	24.8	60	1,800	60	2,400	−29	+33	Cut
Cats	Vertebrals	14.5	22.4	52	1,352	48	1,056	+54	−21	35	20	52	1,560	56	1,940	−43	+24	Inactive (carotids closed)
		24.2	47.6	80	1,920	80	800	+97	−58	47.6	19.5	140	1,960	140	2,380	−59	+21	Inactive (carotids closed)
		40.5	57.7	92	1,840	100	900	+42	−40	57.7	33.7	100	900	76	1,900	−42	+111	Inactive (carotids closed)
		30	55.5	44	1,320	40	800	+85	−40	60	41.6	48	1,200	56	1,680	−31	+40	Inactive (carotids closed)
		33.3	38.4	128	5,120	140	2,800	+15	−45	38.6	23.8	140	2,800	128	7,040	−38	+150	Inactive (carotids closed)
Dogs	Vertebrals	47	50	48	1,680	44	1,320	+6	−21	50	40.5	48	1,440	48	1,584	−19	+10	Inactive (carotids closed)
		50	56.6	40	2,080	40	1,840	+13	−11	41.6	23.1	32	1,760	40	2,600	−44	+48	Inactive (carotids closed)
		21.2	23	64	3,200	44	2,420	+8	−24	57.7	35.7	40	1,600	52	2,184	−38	+37	Inactive (carotids closed)

5. Interpretation of results. From the experiments reported in the preceding sections of this paper one conclusion can be drawn with certainty. It is that sinus and aortic reflexes are not the only agency concerned in the respiratory effects of changes in cephalic or systemic blood pressure. The further conclusion that the respiratory center is directly affected by alterations in its blood supply, in the manner stipulated by the hypothesis of Gesell (8), I believe to be justified, for the following reasons: First, the fact that adrenalin apnea occurs in decerebrate animals with sinus and aortic nerves cut localizes the responsible factor or factors in the brainstem and excludes reflexes from the external carotid area, the possible importance of which has been mentioned by Koch (9). Second, the four sets of experiments just reported had only one evident factor in common, that being alteration in blood supply of the center; since the common result was respiratory depression with increase and respiratory stimulation with decrease in flow it is most probable that the respiratory effect was the result of the change in flow. This can be proved only by exclusion of all other possibilities as they arise; all that are known to me have been excluded.

Even though it be true that alterations in cerebral blood flow have direct effects upon the respiratory center, there can be no doubt concerning the ability of reflexes aroused in the sinuses by changes in endosinusal pressure to produce the same sort of respiratory effect. The observations of Moissejeff (10) and Heymans and Bouckaert (2) have been so regularly confirmed by subsequent workers (11) (12) (1) as to prove this point beyond question. It would appear therefore that the respiratory effects of changes in cephalic blood pressure might be due either to sinus reflexes, or to changes in blood supply of the respiratory center, or to both influences combined. If the conclusions which Heymans (4) has drawn with respect to the normal regulation of the vasomotor and cardio-inhibitory centers are applicable to the regulation of the respiratory center, the direct effects of alteration in blood supply must be relegated to the background and regarded as purely pathological, all respiratory effects of changes in cephalic blood pressure under physiological conditions being attributed entirely to sinus reflexes. This would mean that the sensitivity of the respiratory end-organs of the sinuses to changes in endovascular pressure is vastly greater than that of the cells of the center to the changes in their internal environment produced by changes in their blood supply.

This is a question of considerable theoretical importance because it involves the fundamentals of respiratory control. If the reflex factor is all-important and responsivity to changes in blood flow is purely pathological, it follows either that the cells of the center have such a low metabolic rate that only extreme changes in their blood supply have an appreciable influence upon intracellular conditions, or else the cells are very much less

sensitive to changes in their internal environment than has hitherto been supposed. There is a considerable amount of evidence (see 13, 8, 14) against both of these deductions, and even though this evidence is indirect and inconclusive, it can scarcely be abandoned until there is convincing evidence of the necessity therefor. I do not believe that any evidence has as yet been adduced which compels the belief that these reflex influences are at all essential to the regulation of breathing, however important they may be to the regulation of blood pressure; to this there is only one exception.

The exception is the hyperpnea of anoxemia, which appears to be mainly if not entirely reflex in origin (15) (1). The hyperpnea of excess CO_2, on the contrary, is much more central than reflex in origin (15) (1), and the same appears to be true of the respiratory responses to changes in pH of the blood: Heymans and co-workers (15) found that reflexes were not aroused over the range pH 7.1 to 7.6, within which any physiological changes would fall. Consequently the only change which these recent disclosures create in the Haldane conception of respiratory control is an explanation of the differences between anoxemia and CO_2-excess as respiratory stimulants: the former is mainly reflex in action, the latter mainly central. The fundamental feature of Haldane's conception, namely, the exquisite sensitivity of the center to changes in CO_2 tension in the blood (13), is not affected because the action of CO_2 remains central. Furthermore, since respiration responds to extremely slight increases in blood CO_2 tension while relatively enormous reductions in oxygen tension are required to produce a reflex hypernea, it follows that the cells of the center are vastly more sensitive to their most effective stimulus (CO_2) than the sinus chemical organs are to theirs (oxygen-lack). The central organ therefore remains the most highly specialized part of the respiratory mechanism, so far as chemical regulation is concerned.

Apart from this, sinus reflexes to the respiratory center have not been shown to be necessary to any feature in respiratory control. While their effectiveness cannot be denied, they do nothing that cannot be accomplished quite as well without them by some other mechanism. Thus, the production of apnea by adrenalin in animals in which sinus and aortic reflexes were excluded (figs. 5, 6, 7) shows that increase in central blood flow is fully capable of accomplishing the same end-result as the reflexes. The experiments in which the cerebral circulation was under perfusion control (table 1) showed instances of respiratory depression from increases in cerebral blood flow amounting to as little as 6, 8 and 13 per cent of the preëxisting flow. The smallest rises in endosinusal pressure by which respiration was reflexly depressed in my experiments were 12, 16 and 23 per cent of the existing arterial blood pressure. While there is no common ground upon which these two sets of figures can be compared, they indicate

at least that the respiratory center may be quite as sensitive to increase in its blood supply as the respiratory reflex mechanism is to increase in endosinusal pressure. From the standpoint of relative power of the two influences, there is no doubt that the excitant effect of acute anemia can overcome the strongest possible reflex inhibition (fig. 11); also, the respiratory depressant action of increase in central blood supply may be decidedly greater than the reflex inhibition produced by elevation of endosinusal pressure to a comparable level (fig. 12).

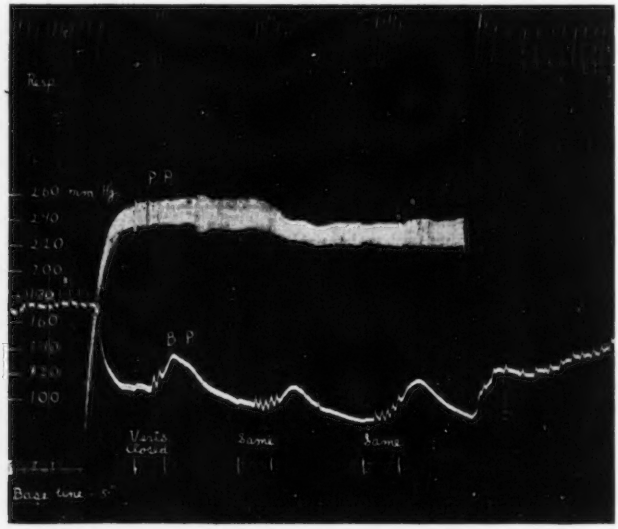

Fig. 11. Acute cerebral anemia overcoming inhibitory sinus reflexes—dog. Barbital: vagi cut; pneumograph; sinuses perfused with defibrinated dog blood; current of oxygen by tracheal catheter throughout—in absence of vertebral occlusion there were no respiratory efforts when endosinusal pressure was kept elevated. Note that each time vertebral arteries were closed respiration and circulation were promptly stimulated.

Drawing thus upon selected examples it is possible to show that Heymans' conclusion—namely, that vegetative centers are entirely unaffected by changes in their blood supply within physiological limits (4)—is inapplicable to the respiratory center. But when the available evidence is considered as a whole, it becomes evident that the direct sensitivity of the respiratory center to changes in cephalic pressure is more variable than that of the sinus reflex mechanism. This is illustrated by the greater difficulty in eliciting adrenalin apnea after sinus and aortic denervation (section 3). On the other hand, the occasional occurrence of typical adrenalin

apneas without the reflexes (figs. 5, 6, 7) clearly shows that the phenomenon is not entirely dependent upon them. Although in some cases the blood flow influence was stronger than the reflex one (fig. 12), in other similar experiments the reverse relation existed (fig. 13).

The explanation of these variations lies, I believe, in differences in tone of the finer blood vessels supplying the respiratory center. This factor must be a determining influence in the effectiveness of a given increase in cerebral arterial pressure, for upon it must depend to a very large extent the effectiveness with which stimulant material can be removed from the

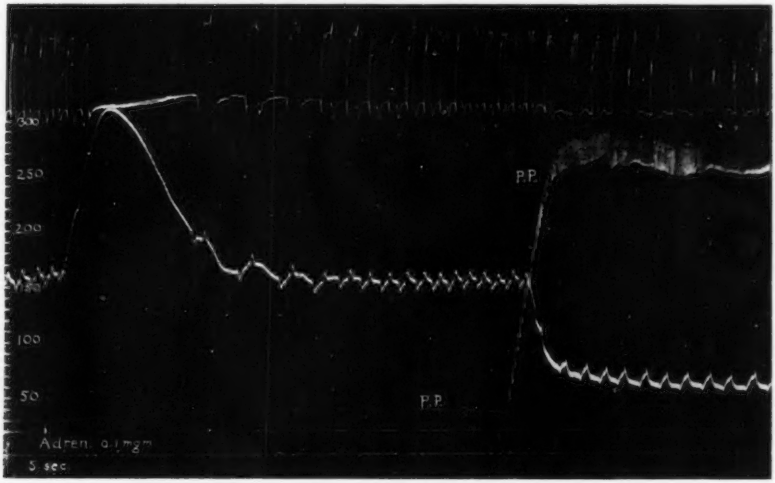

Fig. 12. Comparison of respiratory depressant effects of adrenalin and of high endosinusal pressure. Dog—barbital; vagi cut; pneumograph; crossed-perfusion of both sinuses. Adrenalin injected into femoral vein with pump stopped; after recovery, pump started and resistance adjusted so as to bring endosinusal pressure to approximately the same level as that reached by systemic pressure after adrenalin.

cells of the center. It is this factor, in my opinion, that accounts for the apnea which occurs when blood is readmitted into the previously occluded vertebral and denervated carotid arteries (figs. 1, 2), or when intracranial pressure is suddenly lowered from a high level (figs. 3, 4); for cerebral vessels are known to dilate during a period of acute anemia (16) (5), and when blood is readmitted into them even at the same pressure as before it must exert an unusually marked influence upon the internal conditions of the cells supplied by them. It is possible that under the circumstances which Heymans regards as normal the cerebral vessels are actually in a state of abnormal constriction, so that the vegetative centers are affected little or

not at all by rise in cerebral arterial pressure. While it cannot be denied that adrenalin apnea in the intact animal is at least partly due to reflexes from the circulation, the fact remains that it can also be elicited without the reflexes. I do not believe that the relative parts played by the reflex and blood flow influences in effects of this sort in the intact animal can be determined, even approximately, until more is known about the intrinsic

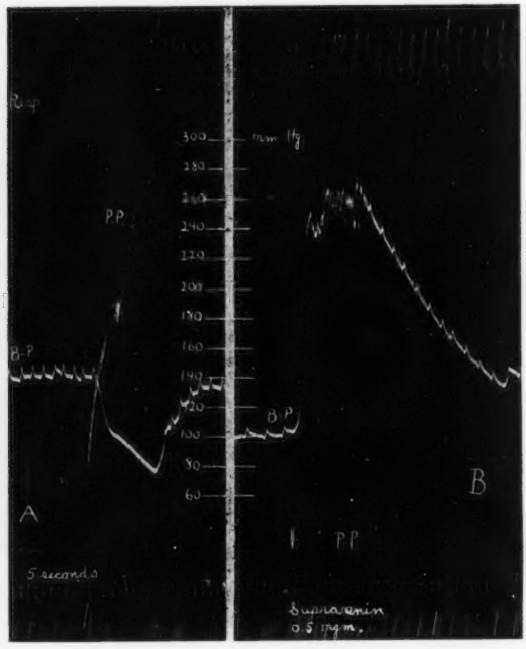

Fig. 13. Same comparison as figure 12. Dog—barbital; vagi cut; pneumograph; sinuses perfused with defibrinated dog blood.

A—effect of elevation of endosinusal pressure to 280 mm. Hg.

B—effect of increase in cerebral blood flow by intravenous injection of suprarenin bitartrate (0.5 mgm.)

regulation of the cerebral circulation. Until then there is no means of defining normal or physiological conditions, and selected examples which prove one point may be countered by others, obtained under other but apparently no more abnormal conditions, which prove the opposite.

There is one other claim, made by Heymans (2) (15), and by Koch (11), which implies that sinus reflexes are necessary to the maintenance of normal respiration in the same sense as they are necessary to the maintenance

of normal blood pressure. It is that the sinus mechanism exercises a tonic inhibitory influence upon the respiratory center. The evidence is the hypernea which follows immediately upon acute section of the sinus nerves or upon occlusion of the innervated common carotid arteries; it is assumed that the latter procedure acts entirely through abolition of tone in the sinus reflex apparatus. Although I have been able to confirm the observations, I believe the above claim should be accepted with considerable reservation, if at all. This is mainly because, even if these hyperpneas are due entirely to removal of a previously exerted inhibitory reflex influence,

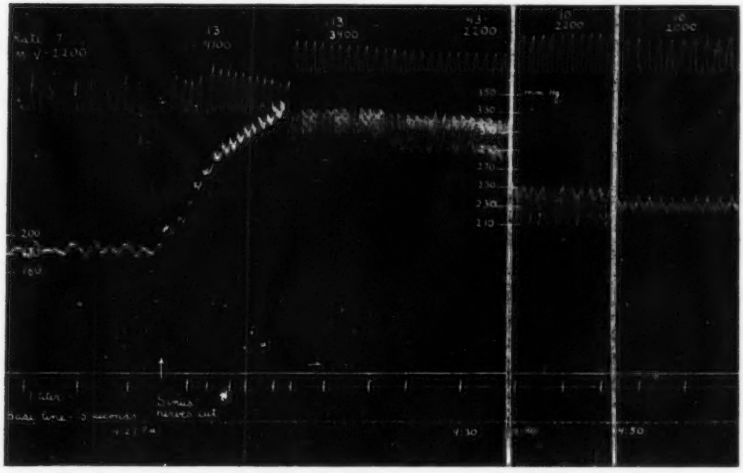

Fig. 14. Respiratory and circulatory effects of acute denervation of sinuses and aorta. Dog—barbital; vagi cut; pneumograph and measurement of expired air, signal showing each liter. At arrow, both sinus nerves were cut: note immediate hyperpnea, with prompt return to same level of ventilation as before. Subsequent tracings follow 10 and 20 minutes later. Sinus denervation permanently removed "apneustic" irregularity of breathing, but did not permanently increase minute-volume.

they are only transitory (1, figs. 1 and 2; fig. 14 of this paper); the hypertension, on the contrary, is much more persistent. It is quite evident, therefore, that tonic inhibition by the sinus reflex mechanism is not nearly as essential to the maintenance of normal breathing as it is to the control of blood pressure: in the case of the respiratory center other mechanisms must be able to assume this function rapidly and completely, which is clearly not the case with the vasomotor and cardio-regulatory centers. It is, however, by no means certain that the hyperpnea produced by section of the sinus nerves or by occulsion of the common carotids is due simply

to removal of inhibitory influences from the sinuses. It was pointed out by Koch (9) that the sinus nerve is a mixed nerve, so that transitory hyperpnea following its section may be due to the inauguration or removal of afferent nerve impulses that had nothing to do with specific sinus reflexes. Furthermore, in all of the recorded examples illustrating this phenomenon blood pressure rose markedly after section of the nerves, so that the condition of the respiratory center might well have been altered in more ways than the simple removal of afferent inhibitory impulses from the sinuses. Likewise in the case of the hyperpnea of carotid occlusion, reasons have already been given (1) for suspecting that it may not always be due simply to inactivation of the sinus reflex apparatus. Since this procedure reduces cerebral blood flow by about 45 per cent (1) it is not unlikely that the effects may be complicated.

For these reasons it seems to me that the discovery of the existence and potency of respiratory reflexes from the carotid sinuses does not alter, in any essential respect, the current conceptions of the mechanism of respiratory regulation. The reflexes seem to be necessary in only one respect, namely, the hyperpnea of anoxemia. Otherwise they accomplish nothing that cannot be accomplished quite as well without them, by alterations in blood supply of the respiratory center. They probably play a part in the respiratory effects of alterations in cephalic blood pressure in the intact animal, but how great or important this part will be must depend to a very large extent upon existing experimental conditions. The state of tone of the blood vessels supplying the respiratory center must be of utmost importance to the blood flow influence, and the sensitivity of the sinus reflex mechanism, peripheral and central, is correspondingly important to the reflex influence. It is quite likely that differences in type and depth of narcosis, trauma, variations in chemical composition of the blood, abolition or inauguration of afferent nerve impulses, etc., all have effects upon one or both of these influences but there is as yet no sufficient evidence to permit even a tentative effort at evaluation.

CONCLUSIONS

1. The respiratory center is shown to be depressed by increase in its blood supply, produced by release of occluded vertebral and carotid arteries, by reduction of high cerebrospinal pressure, by intravenous injection of adrenalin, and by acceleration in cerebral perfusion flow; sinus and aortic reflexes played no part in these effects. In two experiments the vasomotor center was found to behave similarly.

2. It is concluded that the respiratory center is directly affected by alterations in its blood supply in the manner stipulated by the hypothesis of Gesell, namely, because the concentration of chemical stimulant material within its cells is directly influenced thereby.

3. Since the existence and potency of sinus respiratory reflexes, aroused by changes in endosinusal pressure, is established beyond question, the respiratory effects of alterations in cephalic blood pressure may be due either to reflexes, or to alterations in central blood flow, or to both.

4. Reasons are given for believing that under suitable conditions the blood flow influence is quite as sensitive as, and decidedly more powerful than the reflex influence, although the sensitivity of the former is more variable than that of the latter.

5. It is suggested that the effectiveness of the blood flow influence is largely determined by the state of tone in finer medullary blood vessels, and since practically nothing is known about this factor, it is impossible to define normal conditions or to attempt an evaluation of the blood flow influence.

6. It is concluded that sinus respiratory reflexes are essential in only one respect, that being the hyperpnea of anoxemia. Otherwise they accomplish nothing that cannot be accomplished quite as well without them by changes in central blood flow. They are not necessary to the maintenance of normal breathing in the sense that they are necessary to the maintenance of normal blood pressure. The sensitivity of the cells of the center to changes in CO_2 tension or pH of arterial blood is vastly greater than that of the sinus reflex mechanism to any chemical changes in the blood. The central organ therefore retains its position as the most highly specialized part of the nervous mechanism concerned in respiratory control.

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QUANTITATIVE COMPARISON OF SOME MUSCLE AND NERVE REACTIONS AFTER DECEREBRATION AND DECAPITATION

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In two previous communications published in THIS JOURNAL the author has shown that the blood and muscle extracts of various animals, when obtained after decerebration and other injuries to the brain *in situ*, have a greater toxicity than similar specimens taken from the same species after arteriotomy or after complete decapitation (1) (2). From the experimental data presented in the papers mentioned above it was argued that the greater toxicity of blood and muscle extracts obtained after injuries to the brain was due to rapid breakdown of the nervous tissue and consequent formation of toxic bodies carried into the blood stream and distributed to various parts of the body before the heart stopped beating and the circulation ceased. These differences in toxicity were demonstrated by special phytopharmacological methods devised by the author for testing blood sera, tissue extracts and various drugs on plant-physiological test objects or living plants and plant tissues (3). Following these observations it was logical to inquire whether a difference in methods of slaughter would reveal a corresponding difference in zoöphysiological and zoöpharmacological experiments. In this paper the results of such experiments on animals are presented.

METHOD OF STUDY. Experiments were performed, for the most part, on frogs and white rats and occasionally on guinea pigs and kittens. The studies on frogs were carried on in two ways. One method was to study the viability of sensory nerve endings in the skin of the legs, as indicated by the activity of the reflex response of the muscles on dipping the feet in weak acid solutions. In every experiment two frogs of the same species, size and physical condition were selected. One of these was decapitated by cutting through the skull with scissors in the region just behind the eyes. The other frog was carefully pithed with a sharp probe so as to destroy that portion of the brain corresponding to the part removed in the decapitated animal. The sensory reflexes of both legs of the two frogs were next tested by dipping the feet in 0.5 per cent of hydrochloric acid.

Observations were then made to determine the quickness of response and the duration of life in the reflex apparatus of each frog.

In another group of frog experiments, the animals were decapitated and decerebrated in the manner described above; and ten to thirty minutes after the operations had been performed, preparations of the gastrocnemius muscle and sciatic nerve were made, and tested with a faradic current at regular intervals to ascertain the activity of nerve and muscle in each frog until no further response could be obtained with the maximum amount of electric energy.

In experiments on rats, two distinct kinds of experiments were also carried out. Large healthy white rats (*Mus norvegicus*) were selected. Some of these were killed by etherizing the animal and completely severing the head. In other rats, the brain tissue was extensively destroyed under ether by piercing the occiput with a sharp probe and stirring it about in the skull cavity.[1] The hearts of the decerebrated, unconscious animals continued to beat for some minutes after the brain was destroyed. In other experiments, the rats were killed by concussion, or a blow on the head: and in still other studies, instead of being decapitated, the animals were etherized and bled to death by cutting through the vessels of the throat. In a number of special experiments, involving neither decapitation nor decerebration, rats were killed by ether, chloroform, or carbon monoxide. After the rats had been killed by these various methods, two sets of studies were made. In one series of experiments, the activity of the sciatic nerve and muscles of the leg was tested at regular intervals with a faradic current, and the quickness and strength of the responses were carefully noted. These observations were continued until the sciatic nerve no longer responded even to the maximum current available. After death of the nerve the response of the skeletal muscle to direct application of the electrodes was observed, and the final death point of the tissues determined by failure to contract even to the maximal electric stimuli available.

In another series of rats, a different kind of experiment was made. In these, decapitation and decerebration were respectively performed in the manner described above. The *vasa deferentia* were then carefully dissected from connective tissue and kept alive in oxygenated Locke's solution maintained at body temperature. The vas deferens of the rat is an excellent organ for the study of the effect of pharmacological agents on smooth muscle. Vas deferens preparations of exactly the same size and length, but obtained from rats killed under different conditions, were suspended in 50 cc. of oxygenated Locke's solution at body temperature and the height of contraction produced by addition of 0.1 cc. of epinephrine hydrochloride to this medium was determined and measured. As will be seen, a distinct

[1] The word *decerebration* is used in this paper to denote any extensive destruction of the brain.

and marked difference in the degree of contraction was noted after decerebration and decapitation, respectively.

RESULTS. I. *Experiments on frogs. A. Response of sensory nerve endings.* Frogs were decapitated and decerebrated in the manner described above, and the reflex response of the leg muscle to chemical irritation was tested by dipping the foot in 0.5 per cent hydrochloric acid. The quickness of the reflex withdrawal of the foot from the acid solution was noted and the reaction time measured in seconds. The leg was then rinsed in a beaker of water to remove the chemical irritant. Such tests were repeated at intervals of five or ten minutes until the reflex could no longer be elicited or, in other words, the sensory nerve endings were dead. Duration of life in such preparations was carefully noted. In most of the experiments performed it was found that the reflexes died much sooner in the decapitated than in the decerebrated frogs. Thus, for instance, in one experiment the decerebrated frog continued to respond after five hours while the decapitated frog was dead in four hours. In another experiment the decerebrated animal lived three hours while the decapitated frog died in one and a half hours. While the explanation for this difference in reaction between decerebrated and decapitated frogs is not wholly clear, it certainly cannot be altogether due to the loss of blood in the latter because pithing the frogs for decerebration produced nearly as much bleeding as did cutting across the skull.

B. Experiments on nerve-muscle preparations. Nerve-muscle preparations of the sciatic nerve and gastrocnemius were taken from both decapitated and decerebrated frogs and kept from desiccating by applications of physiological saline solution. The response of the sciatic nerve to the electric current in each case was then tested with a Harvard inductorium, and the least energy required to produce a definite response in the nerve-muscle preparations was ascertained every five or ten minutes by adjusting the position of the secondary coil until death of the nerve. The time interval from the beginning of the experiment to this point was carefully noted. Thereafter the skeletal muscle still retained its contractility to direct stimulation by the electric current; and the gastrocnemius was tested by application of the electrodes at frequent intervals until the muscle no longer responded to the maximal stimuli available.

In the majority of such experiments it was found that the duration of life, as tested by the faradic current, was much longer in the nerve-muscle preparations taken from decerebrated frogs than in those obtained from decapitated specimens. The sciatic nerve usually lived from one to two hours longer in the former than in the latter. This difference between decerebrated and decapitated animals was even more strikingly exhibited by experiments on rats.

II. *Experiments on rats. A. Nerve-muscle preparations of rats' legs.*

A large number of rats were killed in different ways and immediately after death the sciatic nerve of one leg (or both) was dissected from the connective tissue and tested for its response to the faradic current. Some of the rats were decapitated; others were decerebrated in the manner described above. Still other rats were killed by severing the vessels of the throat; and in a few experiments the animals were asphyxiated by ether, chloroform or carbon monoxide. The minimal intensity of the faradic current from a Harvard inductorium required to produce a contraction of the leg muscles on application to the sciatic nerve was ascertained and expressed by the distance in centimeters of the secondary from the primary coil. This was repeated every five or ten minutes until the sciatic nerve failed to respond. The electrodes were then applied directly to the skeletal muscles of the leg, and these were likewise tested at regular intervals until they failed to respond to the maximum stimulus available.

A very marked difference was noted in the viability of the nerve and muscle preparations in relation to the method of slaughter. In general,

TABLE 1

DECAPITATED RATS	TIME	DECEREBRATED RATS	TIME
	minutes		*minutes*
Life of sciatic nerve (average of 21 experiments)	52	Life of sciatic nerve (average of 23 experiments)	91
Life of gastrocnemius (average of 20 experiments)	97	Life of gastrocnemius (average of 20 experiments)	123

the sciatic nerve and muscles of the leg lived longer in decerebrated rats than in those which had been decapitated or killed by bleeding or asphyxiation. Thus, for instance, in one experiment, the nerve of a rat, weighing 260 grams and decapitated under ether, continued to respond to electric stimulation for 55 minutes, while the muscle continued to respond for 110 minutes. In another rat of the same weight, decerebrated under ether at the same time, the nerve lived for 110 minutes and the muscle for 130 minutes after death. Table 1 shows the difference between the average figures obtained from all the decapitation and decerebration experiments performed in this connection. The duration of life of both nerve and muscle from animals killed by arteriotomy, or cutting the vessels of the throat, was practically the same as that in preparations from decapitated rats. Thus, for instance, in one experiment after arteriotomy the nerve lived for 60 minutes while the muscles of the leg ceased to respond after 90 minutes. The same was true of preparations excised after death by ether or chloroform. Carbon monoxide, however, produced more rapid death of both nerve and muscle, as tested by the faradic current.

C. Experiments on the vas deferens. The author has used the vas deferens

of the rat extensively for pharmacological testing of the effects of drug and tissue extracts on smooth muscle organs. Thus, Macht and Matsumoto (4) found that this organ is particularly sensitive to extracts of corpus luteum and the degree of contraction produced by such solutions on the vas deferens was an index to the physiological activity of the hormone, running parallel to the results obtained by the vaginal smear method (5). The vas deferens gives a prompt and marked contraction on treatment with epinephrine.

About two hundred experiments were performed on vasa deferentia obtained from rats killed by different methods. The results of these studies indicate a distinct difference in the degree of contraction produced by epinephrine on vasa deferentia from decerebrated rats and those obtained from decapitated animals. When carefully dissected and tested as to their response to epinephrine, the two vasa deferentia from any healthy rat were ordinarily found to give nearly the same height of contraction. Again, when any one vas preparation was tested with epinephrine and then washed thoroughly with Locke's solution, it could be contracted to the same degree by one, two and even three similar successive doses of the drug administered at short intervals of time.

Studies were made on the response of the vasa deferentia obtained from decapitated versus decerebrated rats; and it was found that in 90 per cent of all the experiments made, the contraction elicited by 0.1 cc. of epinephrine was much greater in vasa taken from decerebrated rats than in those removed from decapitated animals. In 10 per cent of the experiments there was either no differenence in the degree of stimulation by epinephrine or, in a few cases, the specimen from the decerebrated animal responded very poorly to the drug. The following figures show the average height of contraction of vasa deferentia taken from decapitated and decerebrated animals, respectively.

	mm.
Average height of contraction by epinephrine of 50 vasa deferentia from decapitated animals	55
Average height of contraction by epinephrine of 50 vasa deferentia from decerebrated animals	87

The effect of arteriotomy, or bleeding without decapitation, was the same as that obtained after complete decapitation of the rats. In all the experiments the organs were lightly weighted so as to obtain the maximum contraction with a lever the short arm of which was 3 cm., while the long arm was 33 cm. In these comparative studies care was taken to make the vas deferens preparations of the same length before beginning the experiments, as measured at room temperature.

COMMENT. The data adduced above, together with the results obtained in many other experiments performed by the author, indicate that the

method of slaughter exerts a definite influence on the reactions of surviving nerve and muscle preparations. This was found to be the case with the sensory nerve endings of the frog's skin, the sciatic nerve and gastrocnemius muscles of both frogs and rats, and with the smooth muscle of the vas deferens of the rat. It was found in each case that an extensive destruction of or injury to the brain prolonged the viability of those organs or tissues, as compared with the duration of life of the same tissues taken from decapitated or exsanguinated animals. The reason for this phenomenon is not altogether clear. It may be partly due to the greater loss of blood in the decapitated animals but the most obvious hypothesis suggesting itself is that the destruction of brain tissue leads to the formation of some products of decomposition which the blood carries around the body before the heart has come to a standstill. Thus, for instance, in case of the vas deferens, such abnormal products of decomposition may be regarded as sensitizing the organ to the effects of epinephrine. This view is supported by the findings obtained from experiments on rats, in which one vas deferens was removed under ether anesthesia, the brain being afterward destroyed *in situ*, and the other organ excised ten minutes later. It was interesting, furthermore, to note that small quantities of thyroxin added to the physiological solution in which the vasa deferentia were suspended sensitized those organs to a subsequent dose of epinephrine; and such sensitization by thyroxin occurred in organs from both decapitated and decerebrated animals. The "decerebrated" specimens, however, were not sensitized by the thyroxin to the same degree as the "decapitated" vasa deferentia. While most of the experiments in the present investigation were performed on vasa deferentia, it is plausible that other surviving smooth muscle organs may also show similar differences in contractility in relation to the method of slaughter. In fact, a number of experiments performed by the writer indicate that the uteri of guinea pigs reveal differences analogous to those of the vasa deferentia from decapitated and decerebrated rats. These findings, of course, are of practical importance in connection with the physiological and pharmacological assay of various drugs. Thus, for instance, in the standardization of preparations of the posterior lobe Roth (6) states that the best results are obtained with virgin guinea pigs which are killed by rapid decapitation. Again, Clemen (7) states that the material used in preparation of thromboplastin is obtained from koshered animals killed by arteriotomy, without stunning, because the stunning of cattle injures the brain tissues, rendering them unfit for the manufacture of this product.

SUMMARY

1. The method of slaughter of certain laboratory animals produces a difference in physiological and pharmacological response of certain surviving organs and tissues.

2. The sensory nerve endings of the frog's skin retain their vitality for a longer period of time in decerebrated than in decapitated animals.

3. Nerve-muscle preparations of the sciatic and gastrocnemius cease to respond sooner to electric stimulation after decapitation than after decerebration or extensive injury to the brain.

4. Surviving vasa deferentia dissected from rats after injuries to the brain respond more powerfully to epinephrine than preparations excised from decapitated or exsanguinated animals.

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THE EFFECT OF METHYLENE BLUE ON HCN AND CO POISONING[1]

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It has long been known that methylene blue increases certain biological oxidations. Meyerhof (1912) found that the O consumption of yeast was raised 200 to 300 per cent above normal by methylene blue. It has been demonstrated by Heymans and Heymans (1922) that a rise in temperature took place in dogs that had received methylene blue injections. They suggest that the effect is probably one of a catalyzer upon the oxidative processes.

More recently Barron (1929) and Barron and Harrop (1928) concluded from experiments on eggs of sea urchins and star-fish, erythrocytes, and mammalian tissues that methylene blue acts as a catalyst in activating O. Furthermore, they found that methylene blue has no effect on increasing the O consumption of those tissues which do not possess aerobic glycolysis, and that inhibition of the respiratory ferment by CN affects in no way the oxidation power of methylene blue. In this connection Thunberg (1918) found that CN does not hinder decoloration of the dye in the system, succinic acid-enzyme-methylene blue, while it does hinder the uptake of O in the system succinic acid-enzyme-O. Wendel (1931) demonstrated that buffered cyanide markedly increases the rate of oxidation of lactate to pyruvate by red blood cells in the presence of methylene blue.

In view of these observations it was thought desirable to see whether such an effect of methylene blue could be demonstrated in living mammals whose aerobic respiration had been interfered with.

Since Warburg (1910, 1923, 1926) has long demonstrated that both CN and CO are specific respiratory poisons, these two gases were used to inhibit aerobic respiration. Warburg (1930) has furthermore shown that the O uptake of red blood suspensions is practically unaffected by CO in the presence of sufficiently large amounts of methylene blue.

It had already been shown empirically in laboratory animals by Sahlin (1926), Eddy (1931) and other workers that methylene blue could be used to

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antagonize the effects of NaCN by injecting it intraperitoneally either before the NaCN injections or immediately afterwards. No work has been done to the writer's knowledge on antagonizing by methylene blue the poisonous effects of inhalation of HCN or CO gases.

For these experiments 80 rats were used. They were divided into groups of 20 each, using that number for each set of control experiments with each gas and for the methylene blue-treated animals with each gas.

The animals were placed one at a time in a chamber containing HCN gas (evolved from the decomposition of NaCN) or CO (about 1 per cent by volume in air, produced by the action of hot H_2SO_4 upon formic acid).

TABLE 1

Average of 80 experiments (20 in each group) showing time during which HCN or CO was administered and time of recovery of control rats and those treated with methylene blue injections

A and C, controls; B and D, treated

CONTROLS		METHYLENE BLUE TREATED	
A	HCN	*B*	
In HCN 3.3 minutes	Recovery 11.2 minutes (100%)	In HCN 3.3 minutes	Recovery 4.0 minutes (36%)
Variation 1 to 7 minutes	Variation 5 to 25 minutes	Variation 1 to $6\frac{3}{4}$ minutes	Variation $\frac{3}{4}$ to $9\frac{1}{2}$ minutes
C	CO	*D*	
In CO 1.1 minute	Recovery 7.5 minutes (100%)	In CO 1.1 minute	Recovery 4.3 minutes (57%)
Variation 25 seconds to $2\frac{1}{2}$ minutes	Variation $4\frac{1}{2}$ to 12 minutes	Variation 25 seconds to $3\frac{1}{2}$ minutes	Variation $\frac{3}{4}$ to $7\frac{3}{4}$ minutes

The gas in the chamber was renewed after each experiment and control. The time elapsing before unconsciousness set in was noted. As soon as an animal became unconscious and gave no further reactions to external stimuli, it was quickly removed from the chamber, and given an intraperitoneal injection of methylene blue. The time of recovery was then determined, using as a criterion of recovery the ability of the animal to run straight forward, since it was found that the use of the hind legs was delayed even when the animal was able to sit up.

The amount of methylene blue injected was 1 cc. of 0.01 M solution in Ringer-glucose solution for each 100 grams of body weight. The results for both HCN and CO are given in table 1.

These results show that the recovery of animals having received methyl-

ene blue is considerably accelerated as compared with the controls. The group averages show that whereas the control and experimental animals lost consciousness in equal times, the times of recovery (taking that for the controls in each case as 100 per cent) were 36 per cent for HCN and 57 per cent for CO. Ringer-glucose injections alone had no effect on the time of recovery. When methylene blue was injected subcutaneously no increase in the rate of recovery was observed indicating that rapid diffusion of the dye was necessary to produce the desired effect.

It would seem therefore from these results that intraperitoneal injections of methylene blue increased certain biological oxidations so that the animals were enabled to recover in a shorter time. The results are consistent with the observations on other materials showing an antagonism between methylene blue and CN or CO. These experiments, however, throw no light upon the question of interpretation of the catalyzing ability of methylene blue, i.e., whether it acts as a catalyst in oxidizing bivalent Fe to trivalent Fe according to Warburg, Kalowitz and Christian (1930), or whether according to Barron and Hoffman (1930) it increases the oxidation of some of the decomposition products of carbohydrate metabolism.

SUMMARY

If rats are made unconscious by inhalation of gaseous HCN or CO, their rate of recovery can be considerably accelerated by intraperitoneal injections of methylene blue. Recovery in the case of HCN required 36 per cent and in that of CO, 57 per cent of the time required for recovery of the corresponding controls.

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STUDIES OF THE LIVER FUNCTION OF DOGS

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The advancement in the study of liver function by the use of dyes has been rapid. Abel and Rowntree (1909) observed that phenoltetrachlorophthalein, injected into the blood, appeared in the bile. Rowntree, Hurwitz and Bloomfield (1913) developed a functional liver test, involving the stool collection of phenoltetrachlorophthalein. Whipple, Peightal and Clark (1913) demonstrated that the intensity of hepatic injury, resulting from chloroform, hydrazine or phosphorus, was inversely proportional to the rate of elimination of phenoltetrachlorophthalein by the liver. McNeil (1916) introduced the use of the duodenal tube for the quantitative study of the excretion of phenoltetrachlorophthalein by the liver. Aaron, Beck and Schneider (1921) adopted a stable preparation of phenoltetrachlorophthalein and modified McNeil's duodenal technique, to study liver function.

Recent investigations (Rosenthal, 1922; Rosenthal and White, 1924; Rosenthal and Bourne, 1928; Rosenthal, 1930; and Rosenthal and Lillie, 1931) have emphasized two points: 1, that the determination, in the blood, of the dye which is excreted by the liver, is a valuable index of hepatic function, and also 2, that bromsulphalein is the most acceptable dye available for the physiological study of the liver.

The bromsulphthalein test for liver function is supported by experimental and clinical evidence. Most of the experimental work with bromsulphalein, as an index of hepatic activity, has been carried out with rabbits. However, Rosenthal et al., (1928, 1930, 1931) determined some effects of anesthetics, alcohol, carbon tetrachloride and fat on the hepatic function of dogs.

In attempting to determine the relation between 1, basal metabolism; 2, calcium and phosphorus content of the blood, and 3, liver function; in both hypo- and hyper-thyroid conditions of dogs, we have observed hepatic function in dogs with normal and damaged livers.

METHODS. The Rosenthal bromsulphalein method was used to test the liver function of dogs in this series of experiments. The bromsulphalein and the corresponding set of color standards were obtained from Hynson, Westcott and Dunning, Baltimore.

Normal dogs. Eleven dogs were used for the normal tests. Two milligrams of dye per kilo body weight were injected into the saphenous vein of the dog tested. After injection, a 4 cc. sample of blood was obtained from the saphenous vein of the opposite leg, with a syringe containing dry sodium oxyalate. Samples were taken each minute during a period of ten

TABLE 1

The percentage retention of 2 mgm/kgm. of bromsulphalein in the blood of dogs

NORMAL DOGS

Serial number	Minutes after injection									
	1	2	3	4	5	6	7	8	9	10
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
2				30		10				
3	100		90	25				20		
4		70	35		15		5	3		
14		80		50	35		12		10	
21	35		25			15		8		5
1	80		45	35	25	12		8		
9	55	40		10	10	9		5		
10	45				10	5			3	
13	90	50	40	20	12		7			
15	50		30				8	3		
11	60	50	40	30	20	10	5	5	0	0
Ave.	64	58	44	29	18	10	7	7	4	3

DOGS WITH HEPATIC CIRCULATION DESTROYED

Serial number	Minutes after injection						
	5	10	15	20	25	30	35
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
9	100	100	99	95	95	93	90
22	95	95	95	90	90	90	90
Ave.	98	98	97	93	93	92	90

CHLOROFORMED DOGS

Serial number	Minutes after injection		
	10	20	40
After 1 day			
	per cent	*per cent*	*per cent*
14	90	80	
21	90	70	
23	95	90	
Ave.	92	80	
After 3 days			
14	90		40
21	80		30
23	80		30
Ave.	83		33
After 5 days			
14	50		30
21	15		5
23	5		0
Ave.	23		12
After 10 days			
14	15	5	
21	15	10	
23	15	5	
Ave.	15	7	

minutes. The samples of blood were then centrifuged and 1.0 cc. of the plasma was divided equally into two small test tubes. Two drops of 10 per cent NaOH was added to one of the test tubes. Two drops of 5 per cent HCl was added to the other test tube. The percentages of the dye in the plasma were read in a bromsulphalein comparator box.

Dogs with hepatic circulation destroyed. Two dogs with normal liver

action, as shown by the bromsulphalein test, were selected. The dogs were anesthetized with ether and injected with 0.1 gram of heparin. A glass T-cannula was used to anastomose the portal vein and inferior vena cava.

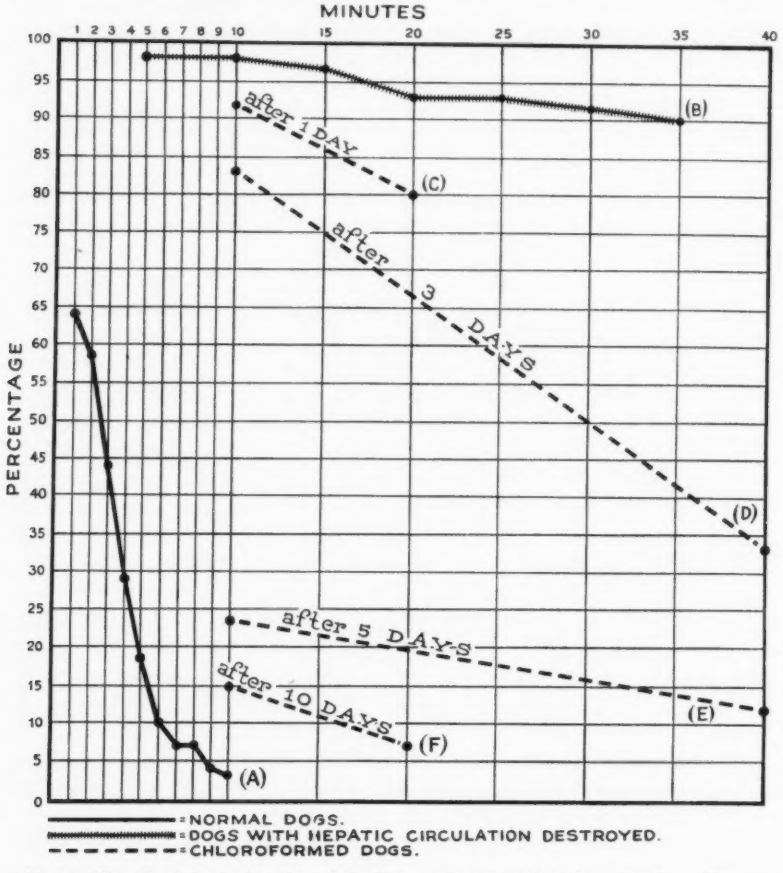

Fig. 1. The physiological action of the liver as indicated by the retention of bromsulphalein in the blood of dogs. The percentage retention of the dye is represented on the ordinates. The minutes, during which the blood samples were taken, are located on the abscissae. The curves show, graphically, the average percentage retention of bromsulphalein recorded in table 1. The letters *A*, *B*, etc., are used for reference in the text.

The hepatic blood vessels were ligated. Samples of blood, to be observed for dye retention, were obtained 5, 10, 15, 20, 25, 30 and 35 minutes after the introduction of the bromsulphalein.

Chloroformed dogs. Three dogs were selected and their normal conditions established. Chloroform anesthesia was then administered for a period of two hours and fifteen minutes. The samples of blood were taken at 10, 20 and 40 minute intervals, following the injection of the dye (see table 1).

The percentages of the dye in the plasma of all the animals were determined by the same method.

Results. Bromsulphalein disappeared rapidly from the blood of all normal dogs. The average retention of the dye in normal dogs, as shown in table 1, was 64 per cent the first minute, and 3 per cent the tenth minute, after the injection of the bromsulphalein. The average percentage retention of the dye in the blood of normal dogs, plotted in figure 1, (*A*), shows a regular decline during the ten-minute observation.

The removal of the dye from the blood of the dogs, after the destruction of the hepatic circulation, was negligible (*B*, fig. 1). The dye, which normally would have been removed almost completely within 10 minutes, averaged 90 per cent retention in the blood of the anastomosed dogs, 35 minutes after the injection of the bromsulphalein.

Chloroform administration caused a pronounced retention of the bromsulphalein in the blood of the dogs. Curves *C*, *D*, *E* and *F*, in figure 1 represent the comparative rates of the disappearance of the dye from the blood of the dogs, after the 1-day, 3-day, 5-day and 10-day rest periods. The highest retention of the dye, in the blood of the chloroformed dogs, was observed the first day after the exposure to the anesthetic (table 1). Ten days after the chloroform injury of the liver, however, only 15 per cent of the dye was retained, in the blood of the dogs, 10 minutes after the injection of the bromsulphalein.

Discussion. Ether anesthesia was used in preparation of the dogs for chloroform administration and in those dogs whose hepatic circulation was destroyed. Slight impairment to the liver function may be attributed to the ether. Rosenthal and Bourne (1928) recorded that, after two hours of ether administration, the 15 minute retention of the bromsulphalein in dogs was about 15 per cent.

Rosenthal and White (1925), by the ligation method, proved that the quantity of the bromsulphalein excreted from the blood of rabbits was dependent upon the amount of functional liver as determined by weight. We demonstrated, by joining the portal vein with the inferior vena cava and ligating all of the hepatic blood vessels in the dogs, that, in dogs without any functional liver, practically none of the bromsulphalein introduced into the blood would be eliminated.

In our work, we observed a relationship of the damaged livers of dogs to the detoxication of nembutal (the nembutal was supplied, complimentary, by Abbott, Chicago). We devocalized some dogs, including two of the chloroformed dogs. The chloroformed dogs, when anesthetized with

nembutal, remained in deep anesthesia, 24 and 36 hours, respectively. The normal dogs recovered from the effects of the nembutal in 2 to 3 hours.

The function of the liver in the detoxication of such substances as nembutal, by normal and damaged livers, offers an important problem for investigation.

SUMMARY

The high retention of the dye in the dogs, after the complete blocking of the hepatic circulation and the union of the portal vein with the inferior vena cava, is further proof that the bromsulphalein injected into the blood is eliminated, specifically, by the liver.

The determination of the removal of the bromsulphalein from the blood of dogs, under normal conditions as well as during chloroform and circulatory injury of the liver, indicates that the bromsulphalein test is a delicate and effective method for the study of hepatic function. Samples of blood obtained 3 and 10 minutes after the injection of the bromsulphalein in dogs provide a suitable basis for the physiological study of the liver.

Dogs which received prolonged chloroform anesthesia and, subsequently, were anesthetized with nembutal, remained in deep anesthesia for 24 to 36 hours; while dogs with undamaged livers recovered from the effects of nembutal in 2 to 3 hours.

We appreciate the interest and helpful criticism of Dr. Edward C. Mason.

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THE CHEMISTRY OF EMBRYONIC GROWTH

III. A Biochemical Study of the Embryonic Growth of the Pig with Special Reference to Nitrogenous Compounds[1]

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The field of embryonic chemistry offers an unparalleled opportunity for the study of life processes. The orderly development of a single cell into a highly differentiated multicellular and multiorganed individual involves a series of chemical syntheses and chemical changes which must eventually be understood before we can truly say that we are familiar with the processes that characterize living matter. The embryologists tell us that the morphological changes which occur during embryonic development represent a recapitulation of the history of the race; may it not well be that by studying the sequence of biochemical changes during embryonic growth we will learn something of the biochemistry of evolutionary processes?

Of the many elements which enter into the composition of the embryo, there is one, nitrogen, that can be readily estimated with a fair degree of precision. Furthermore, by making use of certain characteristic reactions we can determine some of the different chemical groupings and compounds which contain this nitrogen, and these compounds in turn are, in the main, constituents of the proteins, which constitute the bulk of the cellular material. We have accordingly devoted our investigation to a comparative study of the forms of nitrogen in the pig embryo at various stages of its development.

Historical. The first two papers in this series (Gortner (1913, 1914)) dealt with a study of nitrogen changes in the developing eggs of the brook trout, *Savelinus fontinalis* L., and of the giant salamander, *Cryptobranchus allegheniensis*. It was shown that while there was a loss of weight during development, no nitrogen was lost from the eggs although shifts in the various nitrogen ratios did occur. The basic nitrogen increased at the expense of the mono-amino nitrogen and it was suggested that a part of

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the energy of development was derived from the changes taking place in the protein fractions. A definite synthesis of fat from protein was likewise observed.

These early papers included a brief literature review. After the present study had been completed and, together with an extensive literature review, was largely in manuscript form, the recent comprehensive monograph by Needham (1931) on *Chemical Embryology* became available. This monograph is so complete and so excellently written that the reader may be referred to it for the historical treatment.

Suffice it to say that except for a few papers dealing with the stage of embryonic growth at which enzymatic activity becomes apparent, there are very few papers dealing with the biochemistry of the developing mammalian embryo, and still fewer dealing with its nitrogenous constituents. Almost the only papers in this field are those by Buglia and Costantino (1912). These authors studied the nitrogen fractions in the musculature of cattle embryos at various stages of development in comparison with similar fractions in the adult animal. They find that as growth and development progresses there is an increase in the percentage of nitrogen in the tissues (11.31 to 15.05 per cent), a decrease in the "ammonia nitrogen" (20.40 per cent to 9.14 per cent), an increase in the "formol titratable" nitrogen (49.86 per cent to 66.81 per cent), and, within this fraction, a relative greater increase of the diamino acids than of the monoamino acids. They further show that in the aqueous extracts of the embryonic musculature the monoamino acids predominate (90 per cent) whereas similar extracts of adult musculature are characterized by a predominance (76 per cent) of the diamino acids.

EXPERIMENTAL. *The problem.* A review of the literature disclosed the following points:

1. That no systematic study has ever been made on the mammalian embryo, particularly of the nitrogenous compounds.

2. That no data have been compiled on related studies of incubating hen eggs and developing mammalian embryos, and therefore no fundamental facts exist upon which to base the idea that knowledge obtained in working with the chick embryo, the most convenient system, can be transferred to mammalian development.

3. That the work on nitrogen distribution in chick embryos has been quite confusing. In specific cases the whole egg, including the shell, has been used, while in most instances the entire contents which evidently contained a certain amount of food material in the yolk sac and the excretions of the embryo were employed. These quite naturally gave varied results.

4. The unreliability of the results heretofore obtained can be demonstrated in the two papers of Calvery (1929), in the first of which arginine is reported as at first decreasing and later increasing while in the later publi-

cation it is reported as not changing, while the histidine and lysine results were admittedly undependable.

5. That in spite of the large amount of work done in embryonic chemistry, there is general harmony on only a few points.

We have felt, therefore, that by collecting mammalian embryos at different stages, completely dissecting them free of their membranes, measuring carefully their lengths and classifying them accordingly, we could arrive at some conclusions, which would at least represent only the changes which are taking place within the embryo itself, and might assist in clarifying the literature.

The material. We have chosen to work with pig embryos. In the pig, the duration of gestation ranges for 109 to 123 days (4–4½ months). Usually all the normal embryos of the litter are all in the same phase of development, but however it is a matter of common experience that one does find occasionally embryos that are definitely below or above the average length and are in an entirely different stage. This, however, is the exception rather than the rule, and it can be stated with very few reservations that embryos of the same litter, and embryos the same length from different litters, are in the same phase of embryonic life. The average duration of development from the moment of fertilization until all the parts or foundations of the embryo have come into existence is between 28 to 30 days and represented by the 12 to 15 mm. embryo. This period has been termed the critical period. From this, one can easily see that the total duration of gestation can be divided into four critical periods,—each approximately one month.

METHODS. The embryos, ranging in length from 6 mm. to 240 mm., were collected within ten minutes after the death of the mother. In most cases the specimens were still alive, as evidenced by the continuation of the fetal heart beat. Embryos of 60 mm. or less were dissected from the uterine attachments without rupture of the fetal membranes, while larger specimens were kept under amniotic fluid. This precaution was taken to prevent any possibility of drying before the material could be weighed.

Insofar as was possible, the collections were removed from the amniotic fluid within an hour, dried with filter paper, weighed, and immediately placed in acetone for dehydration. The acetone was changed every 12 hours. In order to insure the immediate access of the dehydrating agent to all parts of the larger embryos, and thus prevent autolysis, the abdominal and cranial cavities were incised, care being taken to avoid the blood vessels.

After 72 hours of this preliminary acetone treatment, the material was ground, and, in a continuous extraction apparatus, further submitted to acetone for an additional 48 hours, at the end of which time it was dried at a temperature of 65°C. for ½ hour and weighed. This weight was tabu-

lated as the "dry weight." An obvious objection to this procedure is based on the fact that acetone, particularly when hot, will extract some lipids. The amount of the latter removed, however, is negligible. After this weighing, the material was extracted for 48 hours with alcohol and anhydrous ether successively, dried again at 65°C., for ½ hour and ground in a porcelain ball mill.

The method used of determining the nitrogen distribution is the Van Slyke (1910) method for the determination of chemical groups characteristic of various amino acids, with certain modifications, as recommended by Plimmer and Rosedale (1925), and Cavett (1932), together with certain minor changes with regard to dilution and apparatus as was suitable to this particular problem. The material was hydrolyzed for 24 hours with 20 per cent HCl.

From the beginning we were fully aware that this powdered embryonic material was not a pure protein, and that it must contain certain inorganic salts, carbohydrates, purine bases and other nitrogenous compounds. We were also cognizant of the fact that the Van Slyke nitrogen distribution was reliable for only pure proteins. Attention was called to this by no other than Van Slyke himself. He stated that the method was designed for use only with proteins not accompanied by other classes of substances, particularly nitrogenous substances, which would obviously falsify the interpretation of the results unless the behavior of the non-protein substances were so accurately known that the proper corrections could be made. Just as a matter of emphasis it might be mentioned that Gortner and Blish (1915) have shown that the presence of carbohydrates under the conditions used for protein hydrolysis (20 per cent HCl) will definitely effect the amount of tryptophane converted into humin. More recently, Hauge (1931) pointed out that the presence of fats, particularly the glycerol radical, increases the acid soluble humin, decreases the amino nitrogen in the basic and non-basic fractions and decreases the arginine nitrogen. Any amines formed during acid hydrolysis will appear in the ammonia fraction. Some of the purines and other nitrogenous substances, such as creatin, that occur in animal tissues, are no doubt broken down into simpler compounds. We have shown in preliminary experimentation that creatin and creatinine, under the conditions used in determining arginine, will contribute quantitatively to the ammonia which is formed. It will be noticed subsequently that certain results are listed in the tables under "arginine," "histidine" and "lysine." Obviously these substances are not pure arginine, histidine or lysine, but merely contain those nitrogen fractions which correspond in their chemical characteristics to the distribution of the arginine, histidine and lysine of pure proteins.

In agreement with the above citations, it might be objected that these other substances present besides the proteins would invalidate the results.

These substances, however, are probably present in all the samples, and inasmuch as all samples were treated under exactly the same rigid procedures, we feel that we are justified in concluding that the results we have obtained are comparable.

Tyrosine was determined by the method of Folin and Marenzi (1929), hydrolyzing the material with 20 per cent NaOH and using either the phenol reagent or sodium nitrite solution to develop the color reaction.

Glutathione was determined by the method proposed by Tunnicliffe (1925). One part of fresh tissue was extracted with 4 parts of 10 per cent trichloracetic acid, the final solution was brought to a convenient volume

TABLE 1

Showing the average weight and the per cent of water, ash, nitrogen and glutathione in pig embryos at various stages of development

LENGTH OF EMBRYO	NUMBER OF EMBRYOS	AVERAGE WEIGHT PER EMBRYO	WATER	ASH		NITROGEN			GLUTATHIONE		
				Wet	Dry	Wet	Dry	Ash-free	Wet	Dry	Ash-free
mm.		*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6–7	232	0.313	94.07		*	0.699	13.13	*	0.062	1.04	
10	270	0.500	93.37	0.558	8.43	0.861	12.99	14.18	0.077	1.16	1.26
15	316	0.93	91.38	0.775	9.00	1.061	12.31	13.52	0.119	1.38	1.51
30	158	2.21	91.14	0.708	8.00	1.103	12.45	13.53	0.139	1.56	1.69
50	123	6.55	91.65	1.036	12.41	0.910	10.91	12.45	0.100	1.19	1.35
60	85	14.85	91.05		*	0.966	10.80	*	0.097	1.08	*
80	41	26.00	91.59		*	0.915	10.88	*	0.092	1.09	*
100	20	72.2	91.18		*	0.95	10.78	*	0.079	0.89	*
110	22	82.2	91.02	1.30	14.50	0.972	10.82	12.65	0.078	0.89	1.047
120	15	96.2	91.26		*	0.950	10.87	*	0.078	0.86	*
160	10	238.57	91.71	1.349	16.28	0.891	10.75	12.84	0.068	0.82	0.979
200	7	488	90.34		*	1.014	10.50	*	0.061	0.63	*
240	3	725	88.7	2.58	23.09	1.233	11.01	14.29	0.052	0.46	0.59

Per cent water, 2-4 mm. embryo 97.4 per cent.
Per cent water, 2 weeks old, post-natal pig, 80.2 per cent.
* Groups in which ash was not determined.

and an aliquot taken for the iodine titration, previous tests having shown that practically all of the tripeptide was present in the reduced form.

Total sulphur was determined on the dry material by the Benedict-Denis method (Hoffman and Gortner, 1923).

Total ash was determined by placing a weighed amount of dried material in silica dishes and heating in a muffle furnace at 600°C. for 12 hours.

The experimental data. The average data on the 13 series of embryos, ranging in length from 6 to 7 mm. to 240 mm., are shown in tables 1, 2 and 3.

Discussion. From the accompanying tables it is observed that the total nitrogen decreases gradually until the 50 mm. stage is reached, and

then it assumes a level which is more or less constant throughout the embryonic life. This apparent decrease in nitrogenous material is evidently more relative than actual. The high content of nitrogen at the 6 mm.

TABLE 2

Complete Van Slyke analyses of pig embryos at various stages of development together with certain other related calculations (unless otherwise noted the figures are recorded in per cent of the total nitrogen)

LENGTH OF EMBRYO	6-7 MM.	10 MM.	15 MM.	30 MM.	50 MM.	60 MM.	80 MM.	100 MM.	110 MM.	120 MM.	160 MM.	200 MM.	240 MM.
Amide nitrogen	7.51	7.71	7.17	7.13	7.60	7.44	7.20	7.35	7.58	7.08	6.98	7.23	7.46
Humin nitrogen	5.08	6.14	5.82	5.84	5.92	5.48	5.65	6.27	6.99	6.56	6.74	6.41	6.32
Basic nitrogen	38.04	38.79	38.54	38.57	38.35	38.11	38.58	38.61	38.72	38.62	38.19	38.28	38.15
Amino N	16.25	20.02	20.35	21.41	20.73	21.29	21.22	21.00	21.20	21.50	22.74	22.15	22.05
Non amino N	11.79	18.07	18.19	17.16	17.62	17.82	17.36	17.61	17.52	17.12	15.45	17.13	16.10
Filtrate nitrogen	49.97	46.87	47.51	47.83	47.92	48.23	47.60	47.70	47.53	48.25	47.64	47.51	47.38
Amino N	46.46	41.68	43.17	43.96	44.36	44.88	43.39	43.52	43.08	43.82	42.76	42.21	42.58
Non amino N	3.51	5.19	4.33	3.85	3.55	3.35	4.22	4.18	4.44	4.43	4.88	5.30	4.80
Per cent of basic N that is amino N	42.71	51.61	52.80	55.50	54.05	55.86	55.00	54.39	54.75	55.67	59.54	57.86	57.79
Per cent of filtrate N that is amino N	92.97	88.92	90.86	91.90	92.57	93.05	91.15	91.23	90.63	90.81	89.75	88.84	89.86
Arginine N, per cent	20.80	18.87	17.92	16.91	16.87	17.11	16.91	17.16	17.11	16.47	17.48	16.99	16.53
Cystine N, per cent	0.21	0.21	0.25	0.27	0.33	0.37	0.28	0.26	0.27	0.28	0.46	0.32	0.40
Histidine N, per cent	8.51	8.36	7.13	6.73	7.69	6.12	7.01	6.84	7.01	7.17	4.14	4.97	5.42
Lysine N, per cent	8.52	11.35	13.24	14.66	13.46	14.50	14.38	14.35	14.34	14.70	15.84	16.00	15.80
Per cent arginine of basic N	54.67	48.64	46.49	43.84	43.98	44.89	43.83	44.44	44.18	42.64	45.77	44.38	43.32
Per cent histidine of basic N	22.37	21.55	18.55	17.44	20.05	16.05	18.17	16.78	18.10	18.56	10.84	12.98	14.20
Per cent lysine of basic N	22.39	29.26	34.35	38.00	35.09	38.04	37.27	37.16	37.03	38.06	41.47	41.79	41.41
Per cent recovery	100.60	99.51	99.04	99.37	99.79	99.27	99.03	99.93	100.82	100.51	99.55	99.43	99.31

TABLE 3

Showing the per cent of total sulphur and of tyrosine in pig embryos at various stages of development

LENGTH OF EMBRYO	PER CENT OF SULPHUR			PER CENT OF TYROSINE		
	Wet	Dry	Ash-free	Wet	Dry	Ash-free
mm.						
10	0.0194	0.293	0.319	0.120	1.82	1.98
15	0.038	0.442	0.485	0.146	1.70	1.86
30	0.044	0.505	0.548	0.145	1.64	1.78
50	0.045	0.539	0.615	0.085	1.02	1.18
110	0.041	0.456	0.533	0.048	0.548	0.64
160	0.032	0.384	0.460	0.041	0.500	0.59
240	0.043	0.382	0.490	0.051	0.452	0.58

stage, which insofar as development is concerned is fairly well advanced, no doubt indicates that at the stage of implantation the products of conception are essentially pure proteins. As development continues, other substances as carbohydrates, lipins and inorganic salts, are added as con-

stituents of the embryonic framework, increasing the relative bulk of the embryo but decreasing the percentage of total nitrogen. At the point where the nitrogen becomes constant, the embryo is an adult morphologically, and any changes that may occur from this point on are changes in mass rather than any radical changes of organic structure. Apparently at 50 mm., a constant embryonic protein concentration is reached, and by so utilizing the material selectively absorbed by the placenta from the maternal circulation this level is able to be maintained with but very slight loss.

The fact that most of the decrease in nitrogen is relative is borne out by the big drop in total nitrogen between 30 and 50 mm. There is also a big rise in the ash content between these periods, and if the difference in ash is taken into consideration, the percentages of nitrogen approach one another more closely.

To a lesser extent is this decrease of nitrogen actual. A very small amount of protein material may be used for energy, but at these stages, particularly 15 to 30 mm., the placenta attachments are fairly well organized, and the embryo is no doubt receiving its sources of energy from the maternal organism.

It will be observed from the tables that amide nitrogen showed no change whatever, throughout the whole series. The humin nitrogen of the 6 mm. embryo was somewhat lower than for the later growth stages, but from the 10 mm. through to the 240 mm., the humin shows only very slight,—probably insignificant increase.

Surprisingly, the total basic nitrogen was essentially constant throughout; however, the amino and non-amino nitrogen of the bases showed varied distributions. The amino nitrogen increased as the embryonic length increased while the non-amino nitrogen necessarily decreased.

The most remarkable changes occurred in the constituents of the basic fractions. There was a decidedly definite change in the "arginine" content. The nitrogen distributed in this fraction showed a rapid decrease from the 6 mm. stage to the 15 mm. stage, then a more or less gradual decrease until the 160 mm. stage, where there was a slight rise. The most instructive change, however, occurred in the embryos of the first two groups.

Arginine has been considered by Kossel (1896) to act as the nucleus of the protein molecule, and a compilation of existing data for the arginine content of proteins has been collected by Larmour (1928) to show the relationship of the amount of basic nitrogen to the amount of arginine in the molecule, and hence the governing importance of the latter. But in our determinations we have the total bases remaining the same with a significant decrease in arginine, indicating the formation of other bases which are likewise precipitated by the phosphotungstic acid and whose

proportion of the total nitrogen is within the limits of experimental error, practically the same. It has been stated that arginine gives rise to guanidine and this is the precursor of creatin. This would not account for the rapid decrease, however, as the creatin formed would be precipitated by phosphotungstic acid and later contribute ammonia under the treatment to which the "arginine" fraction is submitted.

From the above it is adequately demonstrated that the more embryonic the tissue, the more "arginine" is present. It might be well to mention that here a strange but logical similarity exists between some facts in embryonic chemistry and tumor chemistry. A number of investigators have pointed out that the more malignant a tumor the more embryonic in nature are its cells. It also has been shown that the more malignant tumors certainly contain more arginine. The growth rate of any tissue may be considered as being dependent, at least in part, upon the amount and nature of the substances available for its nutrition. In recent studies on tumor tissue, experimental evidence has been brought forward to show that arginine definitely induces an increase in the rate of growth (Gilroy, 1930). Moreover, it has been observed that the rate of tumor growth in pregnant individuals is considerably slowed, due to the demand of the embryonic tissues for nourishment, which may ultimately be explained by considering this to be a demand for arginine. It is highly probable, therefore, that this amino acid is necessary for the nutrition of any tissue in which cell reproduction is proceeding rapidly. In both instances it may be stated that arginine is required for nuclear synthesis and cell proliferation.

The rapid decrease in the early stages seems to indicate that nuclear synthesis and cell proliferation are taking place at a greater rate than arginine is supplied to the embryo. Indeed, at that period, when the rate of fall of arginine decreases, all the organs or foundations of embryonic organs have been formed (12–15 mm.) and from then on it is only a matter of growth and slight differentiation.

Histidine also shows a definite decrease. As was expected, the largest variations occur in this group, since in the method used the errors tend to accumulate here. If one considers the early work of Ackroyd and Hopkins (1916) he undoubtedly will conclude that arginine and histidine are interconvertible in the body, and the presence of one or the other is essential for growth processes. It has been later shown, however, that they are not necessarily mutually replaceable but that histidine is far superior to arginine.

The researches of Rose and Cook (1925) indicate that histidine is necessary for nuclear synthesis so it, like arginine, decreases during the period of most rapid growth and differentiation. Histidine, along with arginine, is no doubt the chief precursor of the purine bases which, as we know, are synthesized by the embryo.

The lysine fraction showed a pronounced increase. Large variations also occur in this group. The free nature of the epsilon-amino group gives to lysine a unique importance in the protein molecule and renders it probable that this amino acid would be associated in some way or other with whatever intra-molecular change might take place. However, it must be again stated that the increase in this fraction may very well not be due to any important changes in the actual lysine content, since the material was not a pure protein.

There was no significant variation in the cystine as determined. The actual increase was very small indeed while the relative increase was comparatively large as can be seen from the tables.

The attraction of applying evolutionary succession to embryonic life suggests a new comparison. It has been recently reported (Rosedale and Morris, 1930) that there is a decrease in the amount of "histidine" nitrogen and an increase in "lysine" as we go up the animal scale. While the authors do not make the statement, their tables also show a definite decrease in "arginine." It will be recalled that this is precisely the trend of events as they occur in the developing pig embryo. The above results seem to be unique in bringing forward another bit of evidence, that the transitory changes in embryonic life seem to possess recapitulatory significance.

The nitrogen of the filtrate from the bases shows a sharp decline from 6 mm. to the 10 mm. stage and then assumes a more or less constant value. The amino nitrogen of the filtrate shows a general tendency to decrease while the non-amino nitrogen increases.

It is interesting to note the relationship of the various basic fractions to the total basic nitrogen. The "arginine" fraction, forming 54.67 per cent of the basic nitrogen at the 6 mm. embryo stage, only represents 43.32 per cent at the 240 mm. stage; the "histidine" fraction, forming 22.37 per cent of the total basic nitrogen at the 6 mm. stage, only forms 14.20 per cent at the 240 mm. stage; while, on the other hand, the "lysine" fraction, showing only 22.39 per cent at 6 mm., increases to 41.41 per cent at 240 mm.

The variations of the tripeptid, glutathione (glutamyl-cystinyl-glycine) were very striking. This substance showed a steady rise reaching a peak at about 30 mm. and then decreasing regularly again. The almost universal distribution of this oxidation-reduction system seems to indicate it must be of some vital importance to the cell. However, its content in any one tissue has not as yet been correlated with the predominance or comparative absence of any one type of metabolism and the evidence seems to be very meager for the statement that the greater the glutathione content, the greater is the rate of tissue respiration. In fact experiments have been performed which show that there is no change in reduced glutathione during normal tissue respiration.

Glutathione occurs in the cells largely in the reduced form, yet knowledge of what reduces it remains much as it was when Hopkins (1921) first announced that the disulphide form of glutathione was reduced to the sulphydril form when incubated with kidney, liver or skeletal muscles.

The part played by the tripeptid glutathione in the life of the cell is still obscure in the extreme, and it is quite possible that it is connected with protein metabolism rather than with tissue respiration. It is observed that it rises during the most rapid decrease of total nitrogen, arginine and histidine. It rises during the early embryonic life,—that active period of nuclear synthesis and cell differentiation. When things seem to have reached a more or less stabilized condition, the percentage of glutathione falls. This decline after the 30 mm. stage might be accounted for by assuming that there is at this period a protein fixation of the tripeptid. This fixation may involve glutathione as an intact molecule or its splitting up into pyrrollidone carboxylic acid and cysteinyle-glycine; the dipeptid being fixed to the protein molecule while the pyrrollidone carboxylic acid presumably is the first step in the synthesis of proline and oxyproline which may be later utilized in the formation of hemoglobin.

Total sulphur presents another interesting trend. There is a sharp increase reaching a peak at 50 mm., followed by a gradual decline.

In the 10 mm. embryo the percentage of ash is already 8.43 per cent which indicates how rapidly the embryonic tissue is accumulating inorganic materials. At the 15 mm. stage there was a slight rise, of only about one-half per cent, followed by a fall at the 30 mm. stage to approximately the original level. This fluctuation, although small, was found to be consistent in the several samples examined, and probably indicates the more rapid building up of organic molecules than inorganic materials. This decrease in ash occurs while the glutathione content is at its peak. Between 30 and 50 mm. there was a rapid deposition of ash probably due to the beginning of skeletal development, while between 50 and 240 mm. there was a gentle but regular increase

The play of color shown in the various ashes was surprising. The 10, 15 and 30 mm. ashes were a pale blue; the 50–110 mm. ashes were white, while the 160–240 mm. ashes ranged from a faint pink to a brick red. The red color in the latter stages was presumably due to the iron oxide formed from the iron-hemoglobin complex, but the cause of the bluish tint in the younger stages remains to be determined. These colors, without a doubt, present mute evidence toward certain pronounced fundamental changes in the elementary composition of the various ashes.

The comparison of the total water content of these embryos at different lengths has proven to be quite instructive. In the very small embryo, 2–4 mm. (15 days), the water content was 97.6 per cent. There is, however, some doubt of the accuracy of this figure as representing the water

content of the embryo itself since at these stages it is impossible to separate with any degree of definiteness the extent of the embryo from that of the embryonic adnexa. The per cent of water fell rapidly from this figure to the neighborhood of 91 per cent at 15 mm., at which level it remained, fluctuating within the limits of experimental error, until the 160 mm. stage was reached. Here a second decline began and reached a figure of 88.7 per cent at 240 mm.

It is well known that mammalian embryos during the course of development have, at various times, three types of kidneys; the pronephros, the mesonephros and the metanephros. The pronephros do not function in the higher vertebrates, but it has been quite definitely proven that the mesonephros and the metanephros both are active during the brief uterine sojourn. Now at 15 mm. the mesonephros are very well developed and extend approximately one-half the entire length of the embryo. The relative size of the embryonic kidneys to the entire embryo immediately suggest their importance in fetal life. At this point, where the water content begins to assume a constant level, the mesonephros have, with all probability, begun to function as an excretory organ, controlling the water equilibrium until the 160 mm. stage is attained.

It will be remembered from embryology that the metanephric kidneys are not transformations of the mesonephric type but are of entirely separate, though like origin, springing up and developing entirely independently. In the 100-mm. pig embryo the metanephros does not yet approach one-third the size of the mesonephros, nevertheless they exist side by side, the metanephros growing and developing and the mesonephros remaining constant and finally atrophying when its services are no longer needed. At about 160 mm. the metanephric kidneys, a more discriminating type, presumably take over the excretory function. Their threshold for water is lower than that of their predecessors, and consequently there is a gradual decline in the water level to assume a new equilibrium which is not ultimately established until some time after birth.

As we know, there is a distinct loss of weight in the newborn due to loss of water. This is to be expected since, on the spur of the moment, the animal is confronted with combating three large, hitherto unknown, avenues of water escape; the exposure of a relatively large surface to air of varying degree of saturation, the acts of respiration and the loss by body excretions. We know also that there are drastic anatomical changes accompanying birth, such as the closing of the foramen ovale, the occlusion of the ductus arteriosus, the obliteration of the round ligament of the liver, to say nothing of the partial obliteration of the intra-abdominal umbilical arteries and vein. So it is quite in harmony with the existing circumstances, when we visualize such chemical drastic changes within the cells themselves, whose colloidal material must necessarily attain a new water

level in agreement with terrestial life. This must be a general level determined on the one hand by the water intake as water itself and water acquired as a result of metabolic activities, and water output by cutaneous evaporation, respiration and excretion. This postnatal equilibrium is regulated in the main by the metanephric kidney.

Certain of the results were expressed in terms of the wet embryo, since such computations more closely approximate the conditions as they exist in the living and natural forms. The ash and the sulphur curves are of the same type as presented on the dry basis, showing similar and corresponding rises and depressions.

The results of the total nitrogen are apparently entirely different. From 6 to 30 mm. there is an increase in the percentage of total nitrogen instead of a decrease as shown on the dry basis. Between 30 and 50 mm. there is a slight fall to a constant level which is maintained to the 160 mm. stage, after which there is a continued increase.

In comparing the two series of nitrogen results it appears that while both water and nitrogen (dry basis) show an initial decrease, the decrease in water is so much greater than is that of nitrogen that when calculations are made on the wet basis the curves assume a positive slope. The nitrogen calculated on the dry basis is constant from the 50 mm. stage through the 240 mm. stage. On the wet basis the constancy only persists through the 160 mm. stage, after which there is a slight rise. This rise, however, begins where the total water content begins its second continued decline, and therefore is apparently relative rather than actual.

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SUMMARY

The preceding results were obtained from a detailed study of 1552 pig embryos varying in length from 2–4 mm. to 240 mm. It was observed that:

1. The total nitrogen decreased gradually until the 50 mm. stage was reached and then assumed a level which is more or less constant throughout embryonic life.

2. Amide nitrogen, humin nitrogen and cystine nitrogen showed no significant changes.

3. The total nitrogen of the bases was constant throughout. The basic

amino nitrogen, however, increased while the basic non-amino nitrogen necessarily decreased.

4. The most remarkable changes occurred in the constituents of the bases. There was a decrease in "arginine," a decrease in "histidine" and an increase in "lysine." Most of these changes took place prior to the 30 mm. stage and were quite definite.

5. The nitrogen of the filtrate from the "bases" showed a decrease. Tyrosine, determined separately, also showed a distinct decrease as embryonic life progressed.

6. Glutathione increased rapidly in the early stages reaching a peak at 30 mm. and then declining. It was suggested that glutathione possibly aids in protein synthesis and in the production of proline and oxyproline, which later may contribute to the formation of the hematin molecule.

7. Total sulphur showed a sharp increase to the 50 mm. stage after which there was a gradual decline.

8. The ash content presented an initial increase between the 10 and 15 mm. stages, which was followed by a slight but consistent decrease to the 30 mm. stage. There was a rapid increase in ash from the 30 mm. to the 50 mm. stage and a constant but more gradual increase from this latter stage to the 240 mm. embryo.

9. Total water follows a rapid decrease from the 4 mm. to the 15 mm. embryo after which there is a remarkable constancy which is maintained until the 160 mm. stage is reached. From this latter stage there is a gradual decline in the water content until birth, the final water equilibrium only being reached at an early period in postnatal life.

The idea is advanced that the embryonic equilibrium is maintained by the mesonephros and the second decline only occurs when the metanephros assumes the excretory function.

10. The recapitulatory significance of the variation of the bases was pointed out, "arginine" and "histidine" decreasing while "lysine" increases. This is exactly as the distributions vary as we go up the animal evolutionary scale.

11. The relationship between embryonic chemistry and tumor chemistry was also indicated. The youngest embryos have the highest "arginine" content. This is quite significant, particularly when one correlates it with the fact that the more embryonic a tumor tissue the more "arginine" is found in its contents.

12. And finally, this investigation has brought out the fact that in spite of the constant indirect communication with the maternal organism, mammalian embryos follow certain definite and fixed chemical courses during development which appear to be governed by the inherent nature of the embryo itself and the specific selective adsorption of the placenta rather than any variations in the maternal nutrition, and, from the stand-

point of regularity and gradation of changes mammalian embryos can be studied equally as well as embryos representing comparatively closed systems, with an added advantage of presenting chemical variations distributed over a longer prenatal period.

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STUDIES OF THE INFLUENCE OF PULMONARY MOTION AND DISTENTION ON MOVEMENTS OF THE THORAX

I. Chemical and Nervous Factors Involved in Establishing Apnea

II. The Relation between Pulmonary and Thoracic Movements

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Part I. Chemical and Nervous Factors Involved in Establishing Apnea. Pulmonary ventilation is adapted to the varying needs of the organism by several different regulatory mechanisms. The factors acting on the respiratory center through the circulating blood, so-called chemical regulation, have been studied by physiologists and clinicians during the last decade, while the problems of nervous regulation have been somewhat neglected.

Physiologists, and in particular clinicians, until recently have been satisfied with the Hering-Breuer (1) theory of auto-regulation. According to this conception, the respiratory movements are regulated through the vagus nerves, each distention of the lungs occasioning a centripetal impulse resulting in an expiration, and each collapse a centripetal one resulting in an inspiration. In the physiological literature several experimental observations have appeared which are, however, opposed to this theory and which suggest a different and more complicated nervous control.

Neither chemical nor nervous factors alone regulate respiration; for, as Haldane (2) states, "We cannot separate the nervous from the chemical control of breathing, since each determines the other at every point." It is, therefore, only in combination with chemical factors that the nervous influences can be adequately investigated. For this study we have selected a relatively simple problem, so-called vagal apnea.

It has been frequently demonstrated (Lockenberg (3), Guttermann (4), Head (5), Christiansen and Haldane (6)) 1, that a period of apnea following over-ventilation is prolonged by distention and terminated or shortened by collapse of the lungs; 2, that during normal ventilation, pulmonary distention produces a period of apnea; and 3, that distention of the lungs retards the respiratory rate. These phenomena do not occur after section of both vagi. Because distention of the lungs with nitrogen results in a period of

apnea, just as when the lungs are distended with air, it has been thought that a purely nervous mechanism is implicated. This conception was held at a time, however, when oxygen was still considered to be the most important gas in the mechanism of respiration. Haldane and his collaborators have demonstrated that apnea produced by distention can be prolonged by previous over-ventilation, and shortened by administration of carbon dioxide, and have shown, therefore, that so-called vagal apnea can be influenced by chemical factors. They hold the view that this is not true apnea, but markedly prolonged expiration, and "that the term vagus apnea is an entire misnomer."

In order to learn how great an influence each of these factors, chemical and nervous, has on apnea resulting from distention, it was necessary to choose a definite and readily manifest point or level in chemical regulation. This level is probably represented by the threshold value of the respiratory center, which we define as the level to which the strength of the stimulus in the blood must rise during apnea induced by over-ventilation, before a respiratory movement occurs. The use of this level for this purpose involves the same principle used by Filehne (7) and Knoll (8), and later by Wieland (9), to ascertain the degree of irritability of the respiratory center.

Since the threshold stimulus is not necessarily identical with the usual respiratory stimulus, it was necessary to learn if, and how far, the threshold value of the respiratory center is influenced by pulmonary distention and collapse. To this end a series of samples of blood was taken for analysis during the latter part of, and at the end of, a period of artificially induced apnea.

Experimental procedure. The following experimental procedure was employed. Only dogs were used. The animals were anesthetized with chloralose injected intravenously in a dosage of 0.1 gram per kilogram of body weight, supplemented occasionally with small amounts of the drug if they became restless. A tracheal tube was inserted. The natural minute volume of respired air was measured. Later, artificial respiration was maintained by means of a Starling pump, the stroke volume and rate of which were so adjusted as to give approximately the desired respiratory minute volume. Bilateral pneumothorax was then created. At first this was accomplished by removing at least 6 cm. of two middle ribs on each side. Later, the sternum was split from above downward to the point at which the 6th or 7th costal cartilage was attached. Incisions from the lower end of this opening were then made by cutting laterally along the borders of the 7th ribs. The flaps so made were fastened back. When thoracic movements occurred, the lower intact portion of the thorax was found to move freely and independently of the motion of the lungs. A Y-shaped glass cannula was so introduced into a lateral branch of the deep femoral

artery that the opening lay almost in the main artery. By this device the flow of arterial blood past the mouth of the cannula was free and uninterrupted. Heparin was then added to the dog's blood and the cannula closed with rubber tubes and clamps.

The ventilation was varied by appropriate adjustments in the stroke and rate of the Starling pump in order to bring about both over- and under-ventilation. Distention of the lungs was effected in one of two ways: 1, when sudden distention during a period of apnea was desired, room air was introduced into the lungs of the animal from an air-tight 3-liter glass jar by suddenly distending a rubber bag contained within it; 2, if distention was to be maintained during ventilation, expiratory resistance was increased by submerging the expiratory tube in water to a desired depth. A three-way stopcock inserted into the inspiratory tube permitted collapse of the lungs or the omission of ventilation during one or more strokes of the pump.

The records were made on smoked paper by writing levers. Intra-tracheal pressure was recorded by a Marey capsule connected by a side opening with the intra-tracheal tube. Abdominal respiration was recorded by transmitting changes of pressure in a rubber bag held snugly to the abdomen by a leather cuff to the lever of a Marey capsule. The movements of the lower thorax were transmitted by thread over pulleys to a rod supporting a recording lever and moving in a vertical bearing. The arrival of the piston of the pump making and breaking contact at the end of the expiratory stroke was recorded by a signal magnet.

A number of samples of blood were drawn in rapid succession from the femoral cannula and deposited in test tubes under liquid paraffin by means of a special rotating stopcock.[1] Carbon dioxide and oxygen of the blood were analyzed according to the method of Van Slyke and Neill (10). The pH of the blood was measured by the colorimetric method of Hastings and Sendroy (11).

The volume of air supplied by the pump was regarded as enough to bring about either over- or under-ventilation depending upon whether it was greater or less than the volume breathed spontaneously by each dog after the introduction of the tracheal cannula. Over- or under-ventilation having been attained, artificial ventilation was stopped. The lungs were then either allowed to collapse by removing the expiratory resistance, or were

[1] This stopcock was made of glass and first used in January, 1931. Blood entered through an opening in the bottom of the center plug emerging on the side. Eight perforations through the jacket in a circle at the level of the opening in the center plug lead, by rubber connections, to openings into the lower ends of the glass sample tubes. The sample tubes are held by a stout rubber band against the outer surface of the jacket. The whole apparatus is then filled with paraffin oil. By revolving the jacket with the sample tubes fixed to it, the tubes can be successively and rapidly connected to the arterial needle or cannula.

distended with room air by the method just described. A series of samples of blood was taken toward the end of the period of apnea. To avoid differences in the general state of the animals, the time between the taking of the samples was as short as possible (15 minutes). Because over- and under-ventilation are responsible for marked disturbances in the acid base equilibrium, the periods of ventilation preceding the taking of samples were equal in duration.

The question at once arises: is the sample of blood taken from the femoral artery identical in composition with that arriving at the respiratory center at the same moment? Identity would, it seems, depend on whether the two points are equidistant from the heart, and on whether the velocity in the two arteries is the same. The distance to the femoral artery is obviously greater than that to the respiratory center, but the difference may be compensated in whole or in part by decreased velocity in the precapillary vessels supplying the center. The discrepancy in time cannot be more than 3 or 4 seconds and need not be considered in this connection since the same difference exists both in the state of distention and in that of collapse.

Results. The direct influences of distention and collapse of the lungs on the respiration have already been described. The term distention, or degree of distention, is used to designate only the static factor; that is to say, the state of being distended in distinction to the state of being collapsed. The use of the term is intended to exclude any dynamic factors as far as the lungs are concerned.

When artificial ventilation was stopped with the lungs in *collapse*, thoracic movements began in 14 seconds, with a frequency of 30 per minute (fig. 1a). As soon as artificial respiration was again instituted, the depth and frequency of the thoracic movements decreased. But when artificial respiration was stopped with the lungs *distended* (fig. 1b), apnea lasted 88 seconds and thoracic movements began with a frequency of only 12 per minute. If an animal was slightly *under*-ventilated, it exhibited regular thoracic movements of its own. In collapse of the lungs (fig. 2a) no apnea resulted; the frequency was 24 per minute. In distention (fig. 2b) there was apnea for 15 seconds; thoracic movements then began with a frequency of 10 per minute. After bilateral vagotomy, collapse and distention of the lungs had no influence on the type of thoracic motion (fig. 3). That the dog under description was over-ventilated is shown by the occurrence of apnea before vagotomy; after vagotomy, this phenomenon did not recur. These experiments establish the fact that distention can occasion apnea, or prolong it, if it exists already, and can likewise decrease the frequency of thoracic motion, but only if the vagi are intact. It is also a fact that the length of an apneic period, occasioned by distention of the lungs, can be influenced by the degree of previous ventilation.

The results of the chemical analyses of the blood in all of the experiments show changes in the same direction. Three examples, therefore, suffice.

At the beginning of a period of apnea due to over-ventilation, the oxygen saturation is nearly normal, while the carbon dioxide content is at the lower limit of normal (fig. 4). If ventilation is stopped when the lungs are

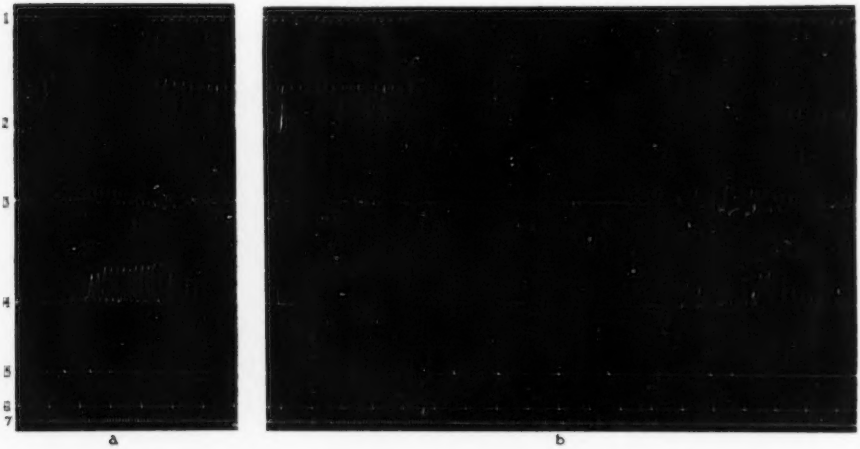

Fig. 1. The effect is shown upon the duration of apnea in a dog which has been the subject of *over*-ventilation when ventilation was arrested *a*, in the state of collapse, and *b*, in a state of distention of the lungs. The curves from above downward are made by:

1. A signal magnet in circuit with the respiratory pump. At the end of expiration, the shaft of the piston made contact.
2. A tambour connected by tubing with the tracheal cannula. Upstrokes of the curve indicate inspiration.
3. A lever moving vertically, connected by string with the lower portion of the thorax. Upstrokes represent inspiration.
4. A tambour connected by tubing with inflated rubber bags applied to the abdomen, to record motions of the abdominal wall. Upstrokes represent inspiration.
5. A signal magnet, to record the time when maneuvers were carried out; as when samples of blood were taken.
6. A time signal every 10 seconds.
7. A time signal every 1 second.

The curves in figures 2, 3, 8, 9, 10, 11 record similar events.

in collapse, the concentration of oxygen decreases and that of carbon dioxide increases, rapidly; when they are distended these changes go on much more slowly. When the lungs are in collapse the first thoracic movement takes place at a much higher oxygen, and much lower carbon dioxide concentration than when they are distended.

Although the concentration of carbon dioxide in the blood of the dog now being described was low, an indication of over-ventilation, apnea did not occur until the lungs were distended during the second part of the experiment (fig. 5). This result might have been due to low concentration of oxygen due in turn to changes in the lungs, the result probably of beginning pulmonary edema. Although pathological conditions were present in this experiment, the various changes in concentration of the gases in the

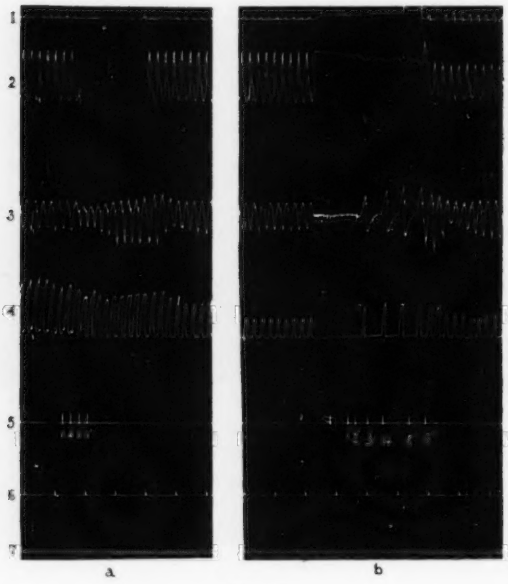

Fig. 2

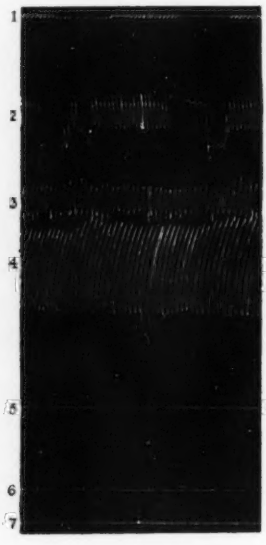

Fig. 3

Fig. 2. The effect is shown upon the duration of apnea in a dog which has been the subject of *under*-ventilation both when ventilation was arrested *a*, in a state of collapse, and *b*, in a state of distention of the lungs. The curves record events similar to those in figure 1.

Fig. 3. The effect is shown of bilateral vagotomy in a dog which has been the subject of *over*-ventilation, when ventilation was arrested, the lungs being in a state of collapse (portion of the curve to the left of the ordinate) and in a state of distention. The curves represent events similar to those in figure 1.

blood at the onset of the animal's thoracic movements, after collapse and after distention of the lungs, were in the same direction as in the first experiment described. Since a period of apnea did not occur during collapse, the threshold value was not obtained. The recorded value for the level of the stimulus of the blood was, of course, greater than the true

threshold value. The difference, therefore, between the threshold stimulus in distention and collapse is really greater than that shown.

Somewhat different results are exhibited in figure 6, obtained from an animal in which the saturation of oxygen at the beginning of the apneic

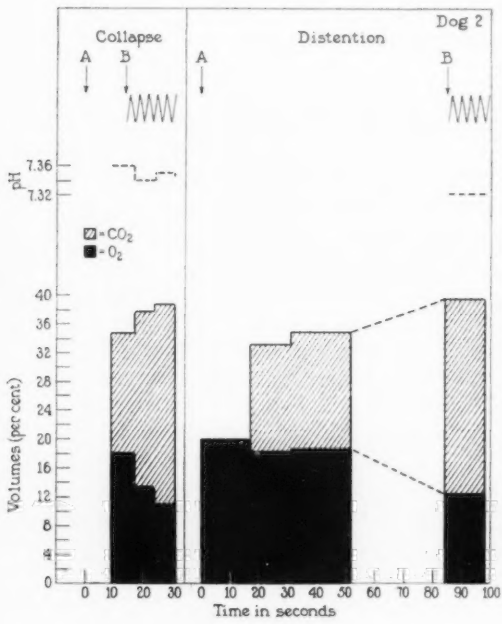

Fig. 4

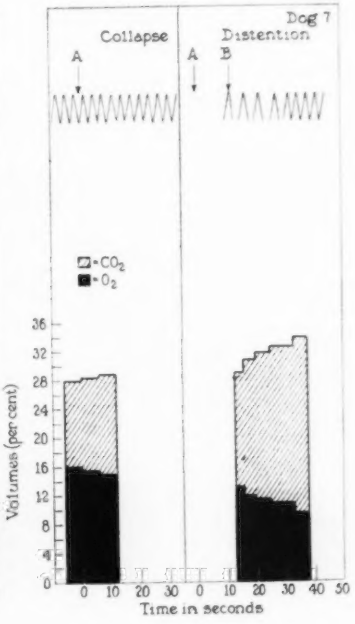

Fig. 5

Fig. 4. Chart of the relation of the concentrations of carbon dioxide and oxygen in the blood of a dog previously over-ventilated by artificial means to the onset of thoracic movements following discontinuance of artificial ventilation with 1, collapse, and 2, distention of the lungs. Dog 2, the same one from which the tracings in figure 1 are taken is the subject of this chart. In this figure and in figures 5 and 6 the arrows at *a* indicate the moment at which artificial ventilation was stopped, those at *b* the moment at which thoracic movements began. The line immediately beneath the arrows shows the periods during which thoracic movements occurred. Apnea was otherwise present. Hydrogen ion concentration of the blood is shown in broken lines.

Fig. 5. Chart showing the same relations as those described in figure 4, but with the dog under- instead of over-ventilated. The tracings in figure 2 are taken from the same animal (dog 7.) It is to be noted that no apnea occurred when the lungs were collapsed.

period was nearly normal. Since there had been over-ventilation, the content of carbon dioxide was low. While the period of apnea lasted only

3 seconds when the lungs were in a state of collapse, the duration when distended was 77 seconds. It is apparent from these three experiments that the changes in the gases of the blood progress more slowly when the lungs are distended than when they are in collapse.

Before passing on to a discussion of these results it is to be noted that in all cases the apnea which occurred during distention was true apnea and not merely prolonged expiration as described by Haldane.

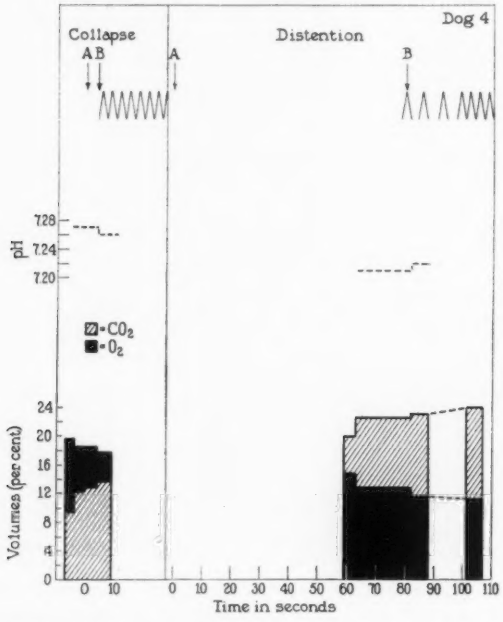

Fig. 6. Chart showing the same relations as described in figure 4 in a markedly over-ventilated animal.

Discussion. If the first thoracic movement marking the end of apnea denotes the moment at which the strength of the stimulus in the blood reaches the threshold value required to actuate the respiratory center, it is apparent, as the result of these experiments, that this value is greater in distention than in collapse of the lungs. The mechanism responsible for this difference may, however, be very complicated. For instance, the medullary centers for the thoracic muscles may change their sensitivity to the impulses coming from the respiratory center. We can only say, therefore, that distention of the lungs has the same effect as decrease in irritability of the respiratory center. Furthermore, following apnea when the

lungs were in collapse, thoracic movements began at a much more rapid rate than when they were distended, which fact strongly suggests that the degree of distention of the lungs has an influence also on the character of thoracic motion.

We can confirm the fact that after bilateral vagotomy, distention of the lungs does not occasion apnea, nor does distention or collapse any longer influence the rate of the thoracic movements. From vagotomy experiments such as these, many authors have concluded that the centripetal impulses produced by distention and by collapse of the lungs run in the vagus. This conclusion is not justified as long as other nervous communications exist (as in the Plexus vagi).

The appraisal of this form of experiment is difficult and requires great caution because the removal of vagal influence changes the sensitivity of the respiratory center to other stimuli such as that of carbon dioxide. It might, for instance, become less sensitive to impulses coming to it through other nervous pathways. This possibility has already been discussed by Breuer. He says "One could suppose that the peripheral stimulus produced by lung distention acts on the medulla as before, and therefore does not go by way of the vagus; but the respiratory center has become too insensitive, too insufficiently labile, for stimuli to produce marked changes in respiratory rhythm." Breuer thinks that this theory is unlikely, although no experiments have been performed which exclude the possibility of such a mechanism. It is therefore necessary for the present to conclude no more than that the degree of distention of the lungs has an effect on respiration only when the vagi are intact.

There remains for discussion the question as to how the centripetal impulses due to distention of the lungs arise. That intra-thoracic pressure is of no importance, as Breuer has already shown, becomes apparent from the fact that no changes occurred in our experiments since the thoracic cavity was in open communication with the atmosphere. It is more difficult to decide whether distention of the pulmonary tissue itself or change in intra-alveolar pressure acts as the stimulus, since these two, being interdependent to a marked degree, can probably not be separated. Since only the difference between intra- and extra-alveolar pressure can be effective, to test the influence of respiration under negative pressure is of no avail. The possibility that centripetal impulses result primarily through the influence of distention of the lungs on the extra-pulmonary circulatory apparatus, complicates the problem; the reference to this possibility is due to the fact that in recent investigations it has been learned that changes in blood pressure influence the respiration (12), (13). Heymans has shown that a rise in blood pressure in the carotid sinus may be accompanied by the occurrence of apnea. In the course of these experiments marked dilatation of the right ventricle, suggesting impairment of the circulation, was ob-

served during a period of over-distention of the lungs. Under this condition the systemic blood pressure and, therefore, the pressure in the carotid sinus seemed to have decreased and yet decreased pressure renders the participation of this mechanism in apnea during distention unlikely. Further investigation of this problem is necessary.

If the degree of distention of the lungs exerts important influences on the behavior of the respiratory center, it becomes necessary to keep the volume of the lungs constant in experiments dealing with the regulation of respiration. How sensitive this aspect of the whole mechanism may be is suggested by the fact that functional residual air (Binger) (normal capacity, Rohrer), a factor in establishing the degree of distention, varies with position. It is greater in the upright than in the prone position and this may be sufficient to change the irritability of the respiratory center, and hence the character of respiration.

SUMMARY

1. In dogs, when the chest is laid open, the influence of distention and collapse of the lungs on the duration of the period of apnea has been studied. By taking successive small samples of arterial blood toward the end of the period, the threshold value of the respiratory center was ascertained.

2. Distention of the lungs is responsible for the onset of apnea or prolongs this state if it exists already, and slows the respiratory (thoracic) rhythm.

3. In distention, respiratory movements do not occur until the oxygen concentration in the blood is lower and the carbon dioxide higher than in collapse.

4. Distention of the lungs may be said, therefore, to influence respiration in the sense of decreasing the irritability of the respiratory center.

5. The influence of pulmonary distention disappears after bilateral vagotomy.

PART II. THE RELATION BETWEEN PULMONARY AND THORACIC MOVEMENTS. Traube (14) has pointed out that distention of the lungs is followed on each occasion by an expiratory movement, and collapse of the lungs by an inspiratory one. This observation has been confirmed several times. Breuer also found that this relation exists provided the individual insufflations are large enough to create appreciable differences in the volume of the lungs. If, however, the insufflations are so small and rapid that no significant change in volume occurs, the thoracic movements are entirely independent of the rhythm of insufflation. This complex or phenomenon was generally regarded as evidence of the auto-regulatory mechanism described by Hering and Breuer. In the literature, and in our own experiments, we find that both in distention and in collapse, regular thoracic movements may occur without any change in pulmonary volume, an

occurrence which is contrary to the behavior one would expect if the Hering-Breuer theory were wholly correct. This study was begun, therefore, in a search for other regulatory mechanisms.

In the preceding part of this paper it was shown that distention of the lungs decreased the sensitivity of the respiratory center, and changed the respiratory rhythm. We wished, therefore, to investigate the extent of the influence of distention of the lungs on the relation between their movement and the motion of the thorax. Observations were first made on the relation of spontaneous movements of the thorax to movements forced

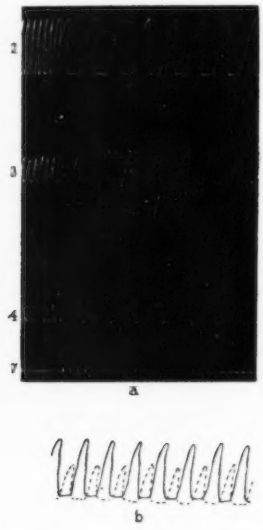

Fig. 7. The time relations are shown during artificial ventilation of a dog. The curves record events similar to those described in figure 1 except that curves 1, 5 and 6 are omitted. In (a) the original curves are reproduced; in (b) a redrawing of curves 1 and 2 in their proper relations.

upon the lungs by varied conditions of artificial ventilation. The general operative procedure was similar to that described in part I.

Results. If the volume of the ventilation is constant, but if the dog is kept *slightly* under-ventilated, the number of movements of the thorax depends on the number of movements of the lungs. Under these circumstances it is easy to demonstrate the relation described by Traube (15) (fig. 7). The motions of the thorax take place in the intervals between insufflations of the lungs. In the experiments if, for example, the ratio of movements of lungs to movements of thorax was, under a certain set of

conditions, 1 to 1, this ratio changed when the number of insufflations, having been 16 per minute, was decreased to 11; there were then two thoracic movements instead of one (fig. 8). When the insufflations were stopped (incident to manipulation of the pump) the rate of the thoracic movements increased. If the rate of insufflations was raised to 16 per minute the 1 to 1 rhythm was resumed at once. A reduction of the rate to 9 again occasioned a 2 to 1 rhythm. Following this slow rate of movement of the lungs, there were found during the periods of collapse, equal and regular thoracic movements (fig. 8).

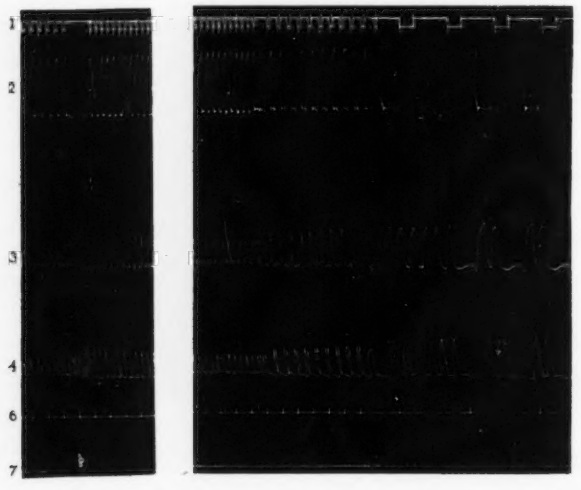

Fig. 8. The effect is shown of changes in frequency of ventilation (strokes of the pump) on the thoracic and abdominal motions of a dog. The division of the curve into two parts indicates an interval of 2½ minutes. At first, reading from left to right, for each stroke of the pump there are two of the thorax. When the strokes of the pump are increased in frequency the ratio of pump to thorax is 1 to 1 preceded and succeeded by short periods when artificial ventilation ceased and a few rapid spontaneous thoracic motions are observed. When the rate of the pump is again reduced, the ratio returns to 1 to 2. Arrest of the pump occasioned once more rapid thoracic motions. The curves record events similar to those described in figure 1 except that the signal magnet for recording the time of maneuvers is omitted.

Following section of the vagi these relations are at once destroyed. If at first a 2 to 1 rhythm is present, this changes immediately after bilateral vagotomy to an unrelated rhythm of about 9 thoracic movements to 6 distentions of the lungs (fig. 9).

Discussion. Our experiments confirm the observation that regular thoracic movements may occur without changes in the volume of the lungs. They also confirm the fact that during each period of insufflation no

thoracic movements need take place. Insufflation acts apparently as an inhibitor of the respiratory center. Attempts to analyze the relations as they are exhibited in our curves, in the light of the Hering-Breuer theory, meet with failure. On examination of the curves one finds that the beginning of distention of the lungs causes an expiratory movement of the thorax, and that an inspiratory motion occurs either at the point of maximum distention or on the arrival of the lungs at the position of collapse. One difficulty is in our curves; artificial expiration is so nearly instantaneous because of the low expiratory resistance, that comparison during this phase is impossible. That the maximum distention should cause an inspiratory movement would be directly opposed to the Hering-Breuer theory, and that return to the position of collapse should likewise stimulate inspiration is unlikely, since the same degree of distention (collapse) would then cause both inspiration and expiration. It seems unnecessary to continue this discussion; it is desirable, however, to emphasize the observation that thoracic movements cease during the active phase of artificial ventilation and take place only during the intervals between insufflations.

So far as the relation of the function of the vagus nerves to these phenomena is concerned, one can conclude no more than that inhibition of thoracic movements during each period of artificial distention of the lungs occurs only when the vagi are intact as vagotomy experiments show.

Since distention of the lungs has been shown to decrease the irritability of the respiratory center, the question arises as to how far, and by what means, the degree of distention controls the relation between movements of the lungs and the thorax. A given concentration of oxygen and carbon dioxide in the blood can either inhibit or give rise to regular thoracic motion depending on whether the lungs are distended or in a state of collapse respectively. Experiments were, therefore, carried out in order to show how, without change in respiratory minute volume, alteration of the volume of the lungs affects the thoracic movements.

If the animal was *markedly* under-ventilated, increase in the volume of the lungs sometimes resulted in bringing about decrease in extent of thoracic movements, but their frequency and their temporal relation to pulmonary movements remained unchanged. Sometimes the changes in diaphragmatic tone described by Head and Hess (16) could be observed. If, however, the animal was so *slightly* under-ventilated as just to avoid apnea, moderate distention produced marked slowing of respiratory movements while considerable distention (fig. 10)[2] produced apnea.

[2] This animal, now being described, under the usual conditions of ventilation moved his thorax only during every second or third instead of during every pause between insufflations. Although the numerical relation between distention of the lungs and thoracic movements was somewhat different, the underlying principle of coördination remained undisturbed, since thoracic movements took place only during the intervals between distentions of the lungs.

With fixed respiratory minute volumes, the effect of increasing or decreasing distention of the lungs on the thoracic movements is dependent in part on the magnitude of the minute volume. The observation that slightly under-ventilated animals become apneic with increased distention of the lungs, may be explained as due to consequent diminished irritability of the respiratory center. If there were a doubt concerning the meaning of these experiments, it would arise from the fact that the blood exhibits

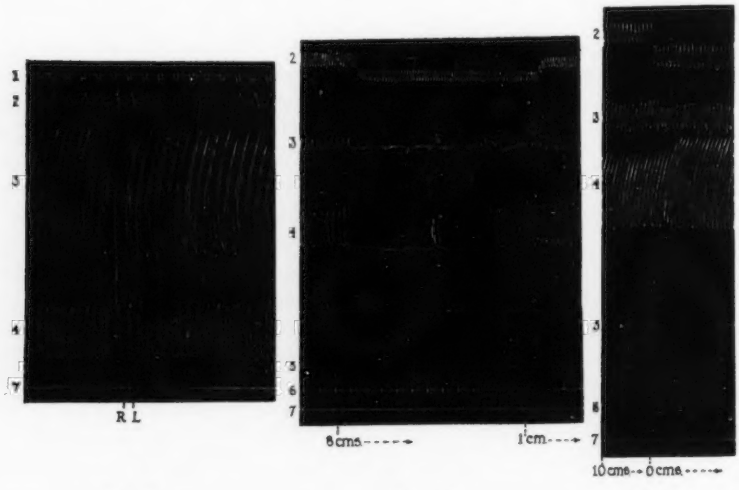

Fig. 9 Fig. 10 Fig. 11

Fig. 9. The effect is shown on pulmonary and thoracic motions of cutting the vagus nerves. The curves record events similar to those in figure 1, except that the signal magnet to record maneuvers and the ten-second signal are omitted.

Fig. 10. The effect is shown 1, of increasing the resistance to expiration to 8.0 cm. water, and 2, of decreasing to 1.0 cm. water. The curves represent events similar to those in figure 1, except that curve 1 representing strokes of the respiration pump is here omitted and that, in the topmost curve in this figure (curve 2) inspiration is a downstroke.

Fig. 11. The effect is shown on pulmonary and thoracic motions of reducing suddenly the resistance to expiration, and so the degree of pulmonary distention, from 10.0 cm. to 0.0 cm. water. The curves represent events similar to those in figure 1 except that the signal for the respiration pump is omitted. In the topmost curve (curve 2) inspiration is an upstroke as in figure 1.

slightly greater oxygen and slightly lower carbon dioxide concentrations during periods of distention of the lungs than during control periods, the respiratory minute volume remaining the same. Because apnea occurs at once, however, it seems unlikely that change in the blood is the decisive factor in bringing about the period of apnea.

Finally, experiments were designed to show whether thoracic movements depended on the extent of the movements of the lungs. Animals, prepared as usual, were much under-ventilated. By means of markedly increased artificial expiratory resistance, great increase in the volume of the lungs was obtained.[3] The expiratory resistance was then so greatly reduced, the respiratory minute volume being kept constant, that the maximum volume of the lungs in inspiration was smaller than the minimum volume had been during expiration (fig. 11). Since, when the lungs were distended, the expiratory phase failed to inhibit the respiratory center, it seemed likely that when they were smaller in full inspiration than they had been before during expiration, the degree of their distention would fail to influence the respiratory center and so bring about dissociation between motions of the lungs and motions of the thorax. But this result did not occur. The motions of the lungs and thorax remained associated, even as they had been before (fig. 11).

This experiment is valuable because although the efficiency of the same respiratory minute volume would be slightly lowered by decrease in volume of the lungs, resulting in a tendency toward increase in acidity of the blood, a tendency which would be more favorable for dissociation of pulmonary and thoracic movements, dissociation did not occur. Since these movements continued to be associated it appears that the links in the chain of causation—degree of distention of the lungs, degree of irritability of the respiratory center, inhibition of respiratory movements—are interdependent to a moderate degree only. The static factor, the degree of distention of the lungs, therefore, is not the occasion for sending relevant centripetal impulses to the respiratory center. One can conclude, in consequence, that impulses dependent upon *movement* itself of the lungs, are concerned in this association. Which mechanism, precisely, movement of the lungs sets into operation, whether it is stretching of the tissue itself, the influence of the air stream on the bronchial mucous membrane or even periodic changes in pulmonary blood pressure, is unknown. The stronger of these unrecognized influences was called into play in some of our earlier experiments (fig. 1b). After the acidity of the blood had risen during apnea above a point where it was certain to stimulate the respiratory center, it was effective in initiating motion even though the degree of distention of the lungs was great enough, under other circumstances to inhibit thoracic movements altogether. Associated motions began again immediately when the lungs were set in motion artificially (though the degree of their distention was still great). No conclusion more searching can be

[3] From the curve recording intratracheal pressure, inferences regarding the volume of the lungs can be made directly. Following over-distention, a return to a smaller size would mean that the initial elasticity had been recovered. Under these conditions the volume for identical pressures would be less.

drawn than that these influences, the influence of acidity of the blood and that of motion of the lungs, are more profound than centripetal impulses arising from the degree of distention. That this inference is just is fortified by experiences with animals in which, though so markedly under-ventilated that distention of the lungs was executed without exhibiting its usual inhibitory influence, complete association persisted nevertheless.

CONCLUSIONS

1. In dogs with widely open pneumothorax, the association of inspiratory and expiratory movements of the thorax and distention and collapse of the lungs results from inhibition of the respiratory movements during each insufflation of the lungs.

2. This association cannot be fully explained by inhibitory impulses arising from the distended lungs, for their state as shown in part I of this paper is able to occasion apnea only under certain conditions.

3. Motion of the lungs or, more properly, one of the factors of motion, stretching of lung tissue, changes in alveolar tension or pulmonary blood pressure, motion of the air in the bronchial tubes, appears necessary for this association.

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REFLEXES IN SPINAL STANDING OF THE CAT[1]

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It has been shown that chronic spinal cats exhibit extensor tonus which is sufficient to support the weight of the body in the standing posture (Ranson and Hinsey, 1930). Observations made upon intact limbs (Ranson and Hinsey, 1930; Hinsey, Ranson and Zeiss, 1931) suggested that this spinal standing was subserved not only by the shortening reaction (Sherrington, 1909) and the stretch reflex (Liddell and Sherrington, 1924, 1925), but also by the positive Stütz or supporting reaction (Rademaker,1926, 1931; Magnus, 1926; Schoen, 1926; Pritchard, 1926). Fulton (1926) has pointed out that there is no reason for considering the shortening reaction as other than a manifestation of the stretch reflex. In this paper the two will be considered under the term stretch reflex.

The positive Stütz reflex consists of contraction of both extensors and flexors of the leg, elicited by applying pressure to the pads of the toes, similar to that of the floor on the foot in standing. The entire limb is fixed in extension so that it resists flexion at all joints. It was seen by Rademaker (1926) in decerebellate dogs and has been studied by Magnus (1926), Schoen (1926), and Pritchard (1926). It was found in thalamic animals but Schoen could not obtain it in spinal animals, although there was a suggestion of it when the extensor tonus was exaggerated by tonic neck reflexes. In addition to this positive reaction, Magnus and Schoen described a negative Stütz reflex which was elicited by passive plantar flexion of the toes. When this was done, the extensor tonus relaxed and the resistance to passive flexion disappeared. It is seen particularly well developed in the decerebrate animal, where it is difficult to demonstrate the positive reaction due to the exaggerated extensor tonus.

In as much as Schoen (1926) did not see the positive Stütz reflex in acute spinal animals, doubt is cast upon the validity of our observations as to its presence in the intact limbs of chronic spinal preparations. Either the spinal standing which we have seen is dependent upon the presence of the stretch reflex, or the "neural balance" in Schoen's preparations was not tilted to the extensor side sufficiently to permit the demonstration of

[1] This study was conducted with the aid of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University.

the Stütz reflex. An endeavor to answer this question has been made in the observations described in this report, where we have analyzed the responses in isolated muscles acting at the knee-joint in chronic spinal animals.

METHOD. Eighteen chronic spinal cats were prepared by transecting in the lower thoracic segments according to the method described by Ranson and Hinsey (1930). In our post-operative treatment, we did not inject salt solution subcutaneously, but forced milk by mouth twice daily. The rectum and bladder were evacuated daily by manual pressure on the abdomen. We had no infections and sixteen of the eighteen animals could stand on the day of the experiment. The animals were kept from one to thirteen days following the transection before the final experiment was performed. Observations were made daily during this period to determine the ability of the animals to stand. They were supported in a hammock made of muslin, which contained four holes through which the legs hung, and were examined for the presence or absence of the positive Stütz reflex, ipsilateral flexion, crossed extension and crossed flexion.

Kymograph records were taken of the isolated extensor quadriceps femoris and the flexor semitendinosus. The animals were first decerebrated by the anemic method of Pollock and Davis (1924). The decerebrations were successful in all but one of the preparations, and the animals remained in excellent condition throughout the course of the experiments. The isolation of the muscles was completed by resecting the insertions of all of the muscles upon the proximal end of the femur, by section of the nerve supply to all the thigh muscles with the exception of the two isolated ones, and by dissecting and denervating the rectus femoris. The animal was then placed in the hammock and the right hind limb was securely fixed so that only the contractions of the two muscles would be transmitted to the levers. This was done by transfixing the distal end of the femur with a drill which was held in place by two upright supports which were adjustable, being fastened by screw nuts at the bottom and by U-clamps into the frame at the top. The proximal end of the femur was clamped as was also the ischium of the pelvis. In some of the experiments a clamp was applied to the fibula just above the ankle-joint. The frame supporting the hammock was screwed to the laboratory table so as to be immovable. The contractions were recorded on the kymograph by means of isotonic levers, with rubber bands used in place of weights.

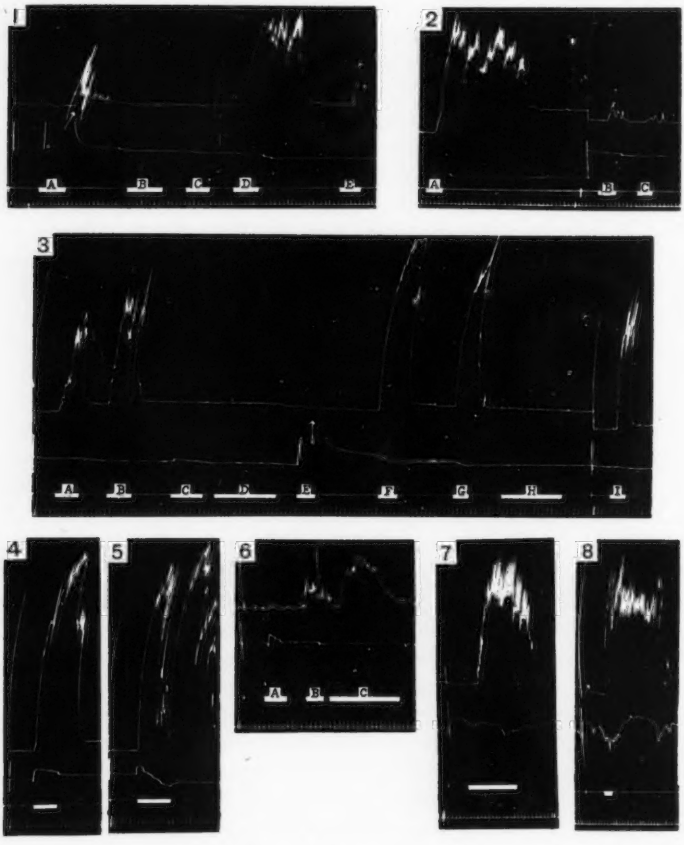
Reflex responses were obtained by applying normal stimulation to the ipsilateral and contralateral hind limbs. For example, it was possible to stimulate for the positive Stütz reflex by gently applying pressure to the pads of the toes and to observe the responses in the thigh muscles. Later on, in some of the experiments, the contralateral tibial nerve at the ankle, the saphenous and the sciatic nerves were exposed and stimulated with

induction shocks applied through shielded platinum electrodes. The ipsilateral tibial nerve at the ankle was also exposed and stimulated. A Harvard coil was used with interruptions at the rate of 50 per second, with both make and break shocks present. The current in the primary was about 2 amperes and the position of the secondary varied from 13 to 6 cm.

There are several advantages to this method. It makes possible a comparison of normal with electrical stimulation. While it is possible to control electrical stimulation in a quantitative way, it is at best unsatisfactory in eliciting reflex responses because the individual fibers in the nerve are activated without respect to the type of sensation they mediate, their normal frequency, or their reflex sign. With the animal in the upright position supported in a hammock, the posture is similar to the normal one and it is as close an approximation as it is possible to obtain and still record the contractions of the muscles. It has been pointed out (Ranson and Hinsey, 1930) that there is a sensitive inhibitory reflex from the skin of the back acting on the positive Stütz reflex, and for this reason an animal on its back would be unsuited for our purpose. Furthermore, the pressure of the hammock upon the skin of the abdomen and groin was found to reinforce the resistance to passive flexion of the leg, a fact which should contribute to the demonstration of the Stütz reflex if it were present in these animals.

RESULTS. *The positive Stütz reflex.* This reflex was not present in its entirety in our preparations for it was not possible to obtain cocontraction of the extensors and flexors acting at the knee-joint by applying gentle pressure to the pads of the toes. This absence of cocontraction was seen at times in the experiments when ipsilateral flexion, crossed extension (figs. 1 and 3), ipsilateral extension and the stretch reflex were demonstrable. Therefore it cannot be said that its absence is due to the poor reflex condition of our animals. Different types of stimulation were utilized, i.e., the slightest pressure to the pads of the toes, greater pressure so as to put the gastrocnemius on the stretch, and maximum passive extension of the gastrocnemius with dorsiflexion of the toes. The paws were also grasped in the position of the positive Stütz and the limb was passively flexed and extended in rapid succession. Bilateral simultaneous positive Stütz was also tried in an endeavor to elicit the response. The responses in the quadriceps and semitendinosus were either entirely negative, ipsilateral flexion was present or ipsilateral extension was obtained, but they never cocontracted in our preparations where there was complete dissection and fixation. Sometimes following ipsilateral flexion, an extensor rebound was seen, both following Stütz positive stimulation and pinching the pad (fig. 1 A).

The importance of complete fixation cannot be emphasized too much. In some of our earlier experiments, we saw cocontraction and believed that



Figs. 1–8. Tracings recorded by right quadriceps (above) and semitendinosus (below). Isotonic contractions. Time in seconds.

Fig. 1. Spinal cat 260, with transection at T 10, five days after operation. A. Ipsilateral flexion from pinching foot. B and C. Absence of positive Stütz reflex on gentle dorsiflexion of toes. D. Crossed extension on pinching left toes and at the same time applying gentle dorsiflexion to the toes of the right foot. E. Crossed extension on pinching left toes without any concomitant stimulation of the right limb.

Fig. 2. Spinal cat 260. A. Crossed extension on pinching left toes and applying gentle dorsiflexion to toes of right foot. B and C. Crossed extension on pinching left toes without any concomitant stimulation of the right limb.

Fig. 3. Spinal cat 270, with transection at T 10, two days after transection. A. Crossed extension on pinching left toes. B. Crossed extension on very strong pinching of left toes. C and D. Stütz positive stimulation to right toes. E. Ipsilateral flexion on pinching toes of right foot. F. Crossed extension on pinching skin about knee-joint. G. Crossed extension on very strong pinching of skin about knee-joint.

we had demonstrated the Stütz reflex in its entirety. However on examination we found that the upright supports were very slightly movable. We then fastened them at the top with U-clamps and the cocontraction was no longer obtained.

Ipsilateral extension. However the absence of cocontraction does not necessarily mean that stimulation of the pads of the toes is without effect upon them in the standing posture. It was possible to demonstrate in five of the animals a definite response in the extensor quadriceps from Stütz positive stimulation. With very slight pressure upon the toe-pads, ipsilateral extension was elicited (fig. 7). Figure 8 illustrates a crossed extension response in this same preparation in which there is a prolonged extensor contraction which appears as an extensor rebound. While ipsilateral extension could be seen very well without previous stimulation, it could also be demonstrated when the stimulation was applied either during the phase of relaxation of a crossed extensor response or immediately after the muscle had relaxed following such a response. In figure 6, Stütz positive stimulation was applied at A, crossed extension was elicited at B, and ipsilateral extension at C. The response at C was obtained by applying slight pressure to the toes of the ipsilateral limb and the contraction is greater in amplitude than the crossed extension. The presence of "direct" as well as "indirect spinal induction" (Sherrington, 1906) may be explained by the assumption that the motor neurones which take part in the crossed extension reflex probably also participate in the ipsilateral extension reflex. "Direct spinal induction" might be explained as due to a central summation and "indirect spinal induction" to an increased excitatory state which is residual following the crossed extension reflex. This latter explanation was given by Creed, Denny-Brown, Eccles, Liddell and Sherrington (1932) to explain the greater readiness of the reflex centers to respond to the stretch reflex after crossed extension. We could obtain no evidence of "negative successive induction" to the Stütz positive reflex following ipsilateral flexion.

H. Stütz positive stimulation again applied. I. Crossed extension on moderate pinching of the toes.

Fig. 4. Spinal cat 270. Crossed response caused by electrical stimulation of the left saphenous nerve. Harvard coil at 6 cm., rapid faradic, about 2 amperes in the primary.

Fig. 5. Spinal cat 270. Crossed response due to stimulation of the left tibial nerve at the ankle. Coil at 10 cm.

Fig. 6. Spinal cat 259, with transection at T 10, two days after operation. A. Gentle pressure applied to right foot-pads. B. Crossed extension on pinching left toes. C. Gentle pressure applied to right foot-pads, showing ipsilateral extension.

Fig. 7. Spinal cat 262, with transection at T 9, five days after operation. Ipsilateral extension on very gentle pressure applied to right foot-pads.

Fig. 8. Spinal cat 262. Crossed extension on pinching left toes.

The ipsilateral extension which was obtained was of a different nature than the extensor thrust which Sherrington (1906) saw in spinal dogs. While his response was of a transitory nature, barely lasting one-half second, the ipsilateral extension which we have seen has lasted several seconds (figs. 6 and 7). It was difficult to obtain, appeared late following the decerebration and occurred in only four experiments. We have never been able to produce pure ipsilateral extension in the spinal animal with electrical stimulation. We have seen cocontraction of the extensors and flexors with electrical stimulation but never extension alone. This is in agreement with the observations of Sherrington and Sowton (1911), Brown and Sherrington (1912) and Ranson and Hinsey (1931). Pinching of the toes in the spinal animal may cause cocontraction while the usual response to both weak and strong stimulation on pinching is ipsilateral flexion and extensor relaxation.

Facilitation of crossed extensor responses. One of the most interesting observations was the facilitation of the crossed extensor responses by applying Stütz positive stimulation to the ipsilateral foot. In figure 1 D, the crossed extension was produced by pinching the contralateral toes with simultaneous Stütz positive stimulation of the ipsilateral limb. The response is quite large in amplitude and long in duration as compared with the contralateral extension (fig. 1 E) obtained without any concomitant pressure on the ipsilateral toe pads. This same comparison may be made in figure 2 between A on the one hand and B and C on the other. This is open to the objection that there is a lack of uniformity of stimulation in pinching the contralateral toe to produce the crossed extension. We endeavored to keep the stimulation as uniform as possible under the conditions of the experiment. Admitting that there was some difference in stimulation, we were convinced that there was no question but that the crossed extension responses were increased in amplitude and duration by applying concomitant Stütz positive stimulation to the limb in which the response was recorded. This may be explained by assuming that the afferent impulses set up by pinching the contralateral toes and those produced by pressure to the ipsilateral toe-pads play upon the same motor neurones or spinal centers and that there is a resulting summation. A similar facilitation to the stretch reflex by the Stütz positive stimulation was observed by Denny-Brown (1929) in the decerebrate animal.

Crossed responses. In this series, crossed flexion was seen to be very well developed in the intact limbs before dissection in only four animals on strong pinching of the toes. In six others, it was present but very difficult to obtain, and then as a rule only at the ankle-joint. In the dissected limb, it was obtained in only three preparations, and in two of these only early in the progress of the experiment. The responses to normal stimulation in the isolated muscles was predominantly extensor in type

with concomitant flexor relaxation, although slight flexor cocontractions were seen on strong stimulation and flexor rebounds were present occasionally.

On electrical stimulation of the tibial at the ankle, saphenous and sciatic nerves, again the responses were extensor, both with weak and strong stimuli (13 cm. and 6 cm. separation of the secondary coil). We did not see the nerve reversals which were present in the gastrocnemius and tibialis anterior muscles in other spinal preparations (Hinsey, Ranson and Doles, 1930). In only one of the eight animals in which electrical stimulation was applied did we observe pure crossed flexion. In the remaining seven when cocontraction was present even on strong stimulation, the response in the flexors was small in comparison with that of the extensors in the same response and with that of the ipsilateral flexion reflex in the same animal.

Strong pinching of the toes is shown in figure 3 B to produce crossed extension and extensor rebound with no flexor participation. On the other hand, electrical stimulation (10 cm. coil separation) of the tibial nerve at the ankle is seen in figure 5 to elicit a cocontraction with inhibition during stimulation and a marked postexcitatory extensor rebound. In figure 3 G, it is seen that there is an absence of cocontraction on strong pinching of the skin about the knee-joint, while figure 4 shows crossed extension with flexor cocontraction from electrical stimulation (6 cm. coil separation) of the saphenous nerve in the same preparation. Comparisons of this nature, together with similar ones in the ipsilateral responses to which mention has been made, show that there is some difference in the responses obtained from normal and electrical stimulation. This is due probably to the massive stimulation of the nerve fibers without respect for their reflex sign and also to the fact that the electrical stimulation is not of a normal frequency.

The more common occurrence of the extensor crossed responses in this series may be attributed to one of two causes or to both. In the first place, the conditions of our experiments favored a "neural balance" tipped toward the extensor side. Sixteen of eighteen of these animals could support the weight of their bodies in standing on their hind limbs before the dissection was started. They were placed upright in the hammock free from extensor inhibiting influences from the back and in a posture favorable to extensor reflexes. In the second place, in this series we were dealing with the extensor quadriceps and the flexor semitendinosus, while in our previous experiments we were recording responses in the extensor gastrocnemius and the flexor anterior tibialis.

We found that the crossed responses developed a great deal as the experiments progressed. For example, cat 270 was decerebrated at 11:15 a.m. At 1:45 p.m., no crossed responses could be obtained. At 2:05, stimulation of the toes was negative for the contralateral side but pinching

the skin over the knee-joint gave crossed extension. At 2:50, the same results were obtained. At 4:20, stimulation of the skin of the toe gave a small crossed extension, that of the skin of the knee a very good one. At 4:40 and 5:00, very good crossed extensions were elicited from both regions. This was seen in other experiments and illustrates the fact that crossed responses recover much slower than the ipsilateral ones (flexion) which were present at 1:45 in good amplitude. Furthermore it shows that the recovery for crossed reflexes differs in the sensory field about the knee-joint from that in the toes.

COMMENT. Schoen (1926) pointed out that there are three essential differences between the positive Stütz reflex and the stretch reflex. 1. Magnus (1926) showed that two types of stimuli may produce it, *a*, proprioceptive elicited by stretching the plantar flexors of the toes, and *b*, exteroceptive by touching the pads of the toes. The stretch reflex is produced only by proprioceptive stimulation. From our experimental evidence, we cannot say what type of stimulation was active in producing ipsilateral extension. 2. Stimulation of the foot in the Stütz reflex calls forth contractions of the muscles of the entire limb, while stretching of the extensor muscle in the stretch reflex elicits a response only in that muscle and there is no spread to other muscles of the limb. The ipsilateral extension we have seen represents a spread from the sensory field in the pads of the toes to the extensor quadriceps acting at the knee-joint. 3. Flexors and extensors as well participate in the positive Stütz reflex while the stretch reflex is present only in the extensor muscles (Liddell and Sherrington, 1924, 1925; Schoen, 1926). We have not seen cocontraction from the Stütz positive stimulation but we have been able to elicit individual contractions of both from pressure on the foot-pads. Thus only a portion of the Stütz pattern could be demonstrated in spinal animals.

We have no observations which are contradictory to the statement of Sherrington (1924), "Elicitation by gravity of the stretch reflex of the limb extensor suggests itself as a basic factor in this static geotropic reflex of standing." These experiments do show however that in addition to the stretch reflex, there are reflexes from the pads of the feet which may reinforce the extensor tonus of the spinal animal. The stretch reflex seemed to be much more resistant to the experimental procedure and was found to be present when the extensor facilitating mechanisms from the foot were not demonstrable. The reinforcement of the crossed extension reflex would be of great value in the extensor phase of the step. Furthermore, ipsilateral extension from the foot should add to the resistance to passive flexion caused by the stretch reflex in standing. This in turn may very well explain the observation that the resistance to passive flexion at the knee-joint from the position of the positive Stütz reflex stimulation is greater than that felt when the force is applied above the ankle in the chronic spinal animal.

In the intact animal, it is highly probable that impulses from the stretched extensor muscles and from the pads of the feet are both active in spinal standing.

It is of interest that Foerster (1927) reports having seen the Stütz positive reflex in two cases of spinal transection in man. Contrary to Rademaker's opinion (1931), we have seen what we have taken to be the Stütz positive reflex in babies, one 2 weeks old, another 8 weeks, and still another 16 weeks old. While Chaney and McGraw (1932) did not look for this reflex, they report that 43.5 per cent of newborn babies are able to support their weight momentarily in the standing posture. This momentary standing may have been subserved in part at least by the Stütz positive reflex.

In the spinal as well as in decerebrate animals, there may be exceptions to the law which says that the ispilateral response is flexion and the crossed one extension. These exceptions in ipsilateral responses require normal stimulation to bring them out and they are difficult to elicit in the spinal animal. It would seem that the spinal cord in spinal animals possesses nearly all, if not all, of the essential mechanisims for subserving tonic activity of a certain degree. While impulses from higher centers may regulate and reinforce the spinal mechanisms and add to them equilibration, their absence in the spinal animal does not make the spinal cord an altogether different field of activity for reflex mechanisms nor deprive it of its ability to subserve a utilizable tonus maintenance in the cat.

SUMMARY

In a series of eighteen chronic spinal cats transected in the lower thoracic segments, decerebrations were performed, using the anemic method. The extensor quadriceps femoris and the flexor semitendinosus were isolated by dissection and the animal was placed upright in a hammock. With the right hind limb securely fixed, kymographic tracings were made of the reflex responses in these muscles. In the same animal, it was possible to apply normal stimuli to the ipsilateral and contralateral limbs and also to use electrical stimulation to the various nerves.

The Stütz positive reflex (positive supporting reaction) with cocontraction of the flexors and extensors of the knee was not elicited on stimulation of the pads of the foot. In some preparations, a portion of the pattern was present as an effect on the extensor responses of the quadriceps, either an ipsilateral extension or a facilitation of the crossed extensor reflex, both in amplitude and duration. At times in the presence of a well-developed stretch reflex in the gastrocnemius, no response could be obtained from Stütz positive stimulation; at other times ipsilateral flexion was seen.

Crossed flexion was observed but the crossed responses to normal and electrical stimulation were generally extensor in type in these muscles acting at the knee-joint.

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CONTINUOUS PANCREATIC SECRETION

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At present it is generally accepted that pancreatic juice is not secreted at all, or at best in only small amounts, during fasting or in the interdigestive periods. Since the work of Claude Bernard, Pawlow, and Bayliss and Starling, the conviction that entrance of food into the digestive tract is necessary to start the pancreatic secretory process has been so strong that the evidence reported by various investigators suggesting a continuous secretion has been overlooked.

Continuous secretion was noted but held to be pathological by Claude Bernard (1), Pawlow (2), Jablonski (3), Walther (3), Babkin (4), (Babkin (5) expresses a somewhat divergent point of view), Tonkich (7), Elman and McCaughan (11). Data or opinions favoring continuous secretion are given by Bylina (6), Ivy and Farrell (8), Ivy (9), Dodds and Bennet (10). Evidence for continuous secretion in various species is also available: comparative review (13), cats (12), rabbits (14), man (12, 15, 16).

In the following study we present data which indicate that pancreatic secretion in dogs is also continuous during fasting.

TECHNIQUE. In order to observe the flow of pancreatic juice we used the method described originally by Rous and McMaster (17) in their studies on biliary secretion. The same technique was adopted by Elman and McCaughan (11) in their studies on the pancreas. This method permits the collection of the total output of the pancreatic juice under most favorable conditions and without bacterial contamination. It differs from the Pawlow fistula in that the minor duct is ligated, thus cutting off all communication with the intestine. It also makes unnecessary the insertion of a cannula to dilate the cicatrizing end of the duct.

Under ether anesthesia, and with aseptic precautions, pancreatic fistulas were established in a series of large male dogs. The major pancreatic duct was isolated first. Then the minor duct was ligated and divided, and the portion of the pancreas between the two ducts was dissected free from the intestine, thus assuring destruction of other accessory ducts in this area. A cannula attached to a rubber U tube was inserted into the major duct and then the latter was divided close to the duodenum. The small

opening in the intestine left by the distal segment of duct was closed by interrupted inversion sutures. A piece of omentum was drawn through the space left between the pancreas and the duodenum and fixed to the latter by sutures in order to prevent the reëstablishment of communications between the ends of the divided ducts. The rubber tube was brought out through the abdominal wall by means of a stab wound just below the costal margin, and the free end was connected to a small glass T-tube which led to a rubber collecting bag. The whole apparatus was held in place by a protective dressing. The bag was emptied at intervals of usually 24 hours and the amount of secretion was measured. The animals recovered rapidly from the effects of the operation. For the first two days after the establishment of the fistula the secretion usually consisted of a small amount of very mucinous material. Thereafter the daily output varied between 200 cc. and 750 cc. and represented essentially normal juice of fairly constant composition. Some of the animals died at the end of 8 days with symptoms associated with acidosis and dehydration. Vomiting was not a pronounced symptom. All dogs used for long time experiments received daily injections of NaCl and $NaHCO_3$ to prevent dehydration and acidosis. They all lost weight, sometimes up to 38 per cent. At autopsy accessory communications between the pancreas and duodenum were looked for, but were never found. The pancreas showed gross and microscopic evidence of shrinkage of the parenchyma, which was probably associated with the loss of fat. The weight of the pancreas decreases during inanition very nearly in proportion to the body weight (18). Diminished turgidity due to lessened resistance to outflow may also be a factor. Anrep (19) has shown by oncometric measurements considerable variation in volume of this organ dependent on outflow. In most instances there was neither evidence of atrophy due to obstruction of the ducts nor of inflammatory changes.

For observations on spontaneous secretion during fasting, animals were deprived of food for periods varying from a few hours to 72 hours before the experiment was started. All precautions were taken to avoid the possible influence of conditioned reflexes upon secretion and the observations were made in a room where they never saw nor received food. They were trained to lie quietly and often slept while the experiments were in progress.

The rate of secretion was studied by connecting 5 cc. pipettes graduated in 0.02 cc. to the rubber outlet tube in a horizontal position, and making readings at suitable time intervals. Observations were made over periods extending from one hour to 3 hours, or even longer, and specimens were collected for analysis.

The "secretory pressure" (20, 21, 22) was determined by measuring the maximum height attained by the column of juice when the duct system was

connected with a manometer tube of 2 mm. bore. A column 100 mm. in height was equivalent to 0.7 cc. of pancreatic fluid. The manometer readings were taken as approximations of secretory pressure.

EXPERIMENTAL RESULTS. *Continuous pancreatic secretion during fasting and the temporary acceleratory effect of secretin.* The following table represents a typical example of continuous pancreatic secretion in an ani-

TABLE 1

Dog 34. Continuous secretion of pancreatic juice

Fistula established October 17, 1930

DATE	LENGTH OF PERIOD	CC. COLLECTED	NUMBER OF CC. PER MINUTE		REMARKS
	hours				
Oct. 18	24	None			
Oct. 19	24	None			
Oct. 20	24	180.0	0.063		
Oct. 21	24		0.063		
Oct. 22	24	75.0	0.052		
Oct. 23	24	130.0	0.090		
Oct. 24	24	200.0	0.139	average	
Oct. 25	24	210.0	0.146	0.143	
Oct. 26	24	120.0	0.083		Fasting
	minutes				
	10	1.28	0.128		Dog fasting 72 hours. No water allowed for 3 hours before readings made
	10	0.428	0.043		
	10	0.856	0.086		
	10	0.286	0.028		
	10	0.143	0.014		
	10	0.143	0.014		
Oct. 28	10	0.214	0.021		
	10	0.143	0.014		
	10	0.643	0.064		
	10	0.572	0.057		
	10	0.856	0.086		
	10	1.07	0.107		
	Average		0.055		

0.055 cc. per minute is equivalent to 79.5 cc. per 24 hours, which is about 26 per cent of the amount secreted before fasting.

mal fasted 72 hours. The average rate of secretion after fasting 72 hours was about one-fourth of the average rate in 24 hour secretions before fasting. We look upon the 24 hour collections in dogs that received food as being made up of the basal continuous secretion plus secretin juice.

In another dog, the rate of secretion decreased more or less continuously during 48 hours of fasting, coming to about one-third of the pre-fasting rate. This type of observation was repeated a great many times with the same result.

It may be well to point out at this juncture that the term "continuous" does not necessarily imply an uninterrupted stream of pancreatic juice during the interdigestive period; pancreatic secretory activity continues in the absence of food stimuli, the rate of flow varying from minute to minute and from hour to hour (see tables). As has been stated by other investigators, the secretion of the pancreas seems to be discharged in spurts when observed in a pipette (see Biedermann, 13). This is analogous to the discharge of urine by the kidney.

After establishing the basal rate of secretion during fasting, the influence of secretin was determined.

When 10 cc. of a solution of secretin, prepared according to the method of Weaver (23) and co-workers, were injected intravenously into a fasting animal the flow of pancreatic juice was increased temporarily. This acceleratory effect lasted about 10 minutes and then the regular fasting rate of secretion was resumed. Marked variations occurred in the rate of secretion in the preliminary period of observation. However, with secretin, the rate increased beyond these variations. The results with secretin in these experiments differ from those of Bayliss and Starling in so far as they represent an acceleration of flow rather than an initiation of secretion. Examination of the data of Bayliss and Starling's acute experiments shows that there was no secretion before the injection of secretin or after the effect of secretin had worn off. This was due to the fact that the observations were made while the animal was under the influence of an anesthetic and other factors which are discussed later.

A parallel study was made of the secretory pressure of the pancreas during fasting and after the ingestion of food. This was repeated on several dogs with similar results; for the sake of brevity we include here only a typical example (see fig. 1, dog 6). Here the maximum fasting pressure attained was 340 mm. One hour and 20 minutes after feeding the pressure curve rose to 390.

So far as we know these are the first figures recorded for secretory pressure of the pancreas in the unanesthetized animal under fasting conditions. While the fasting pressure is of about the same order of magnitude as the bile pressure (20, 21, 22) it rises distinctly after feeding or secretin injection. In this respect the pancreatic secretion differs from bile. The bile secretion pressure is the same before and after feeding (22). The rate of secretion, therefore, does not affect the "secretory pressure." The importance of these facts will be referred to below.

Does continuous secretion represent pathological hypersecretion? At this point it may be profitable to discuss the question whether the phenomenon which we have described is a pathological hypersecretion, as was maintained by the early investigators in this field, and by more recent workers including Babkin (4) and Elman and McCaughan (11). Claude Bernard

and Pawlow attributed continuous secretion to irritation by the cannula in the intubated duct and to infection. Careful dissection of the drainage system at autopsy in our animals revealed that the cannula always lay outside the pancreas, enveloped in a connective tissue sheath which replaced the small segment of duct which originally held the cannula; the latter never encroached upon the parenchyma or the intrapancreatic portion of the duct system. With regard to infection, careful microscopic examination of the pancreas, post-mortem, revealed no evidence of inflammation except for some connective tissue proliferation in the region

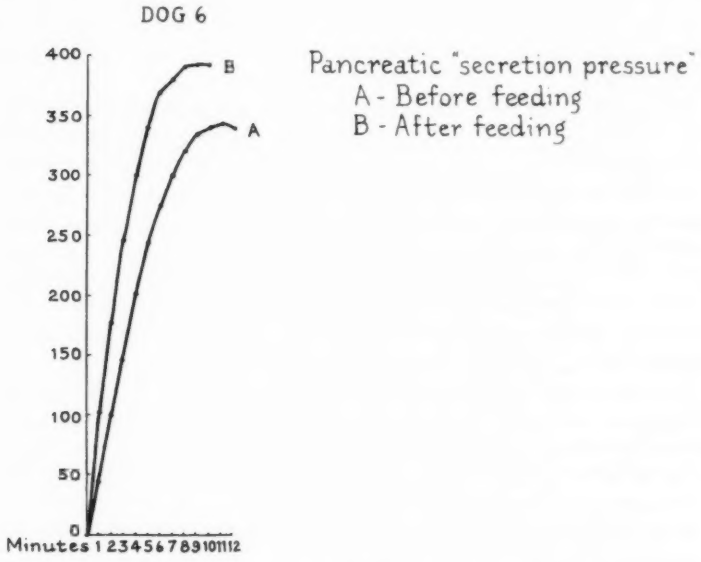

Fig. 1. Manometer readings in millimeters of pancreatic juice

of the dissection, which was done at the time of intubation of the major duct.

Babkin, on the basis of Walther's experiments, brought the continuous pancreatic secretion into relation with a gastric hypersecretion resulting from the pancreatic fistula and more recently Elman and McCaughan again advanced this point of view attributing the spontaneous pancreatic flow to a secretin effect, resulting from the lack of neutralization by the pancreatic juice of the exaggerated gastric secretion. Babkin in his extensive discussion of the subject puts all continuous secretion down as "pathological hypersecretion" and describes the experiences of Jablonski

and Walther in connection with the symptoms, with which we are now familiar, resulting from the *complete* loss of pancreatic juice. It should be noted that Babkin specifically states that in the dogs operated upon by the Pawlow technique the minor duct is left intact, i.e., communicating with the intestine. According to Babkin, conditions are "normal" only when there is a suitable distribution of flow between the duct leading to the intestine and the duct leading to the outside. In Jablonski's dogs there was evidently little or no flow into the intestine. Now since the flow to the outside was large and dehydration and other symptoms occurred, Babkin called this "pathological hypersecretion." He is quite right in saying that for purposes of long observation dogs must be used in which sufficient juice reaches the intestine, but when he calls complete diversion of juice to the outside "pathological hypersecretion," it seems he is begging the question.

The gastric hypersecretion as a possible factor is excluded by observations of Tonkich (7) and Bylina (6). Both observed continuous secretion independently of the possible effect of gastric HCl reaching the duodenum.

To further verify this point under our own working conditions, the following experiments were performed. A 2 per cent solution of $NaHCO_3$ was introduced into the stomach of a fasting secreting animal in amounts far beyond the quantity of alkali which could be supplied by the pancreas in order to neutralize the gastric contents. After the introduction of three successive portions of 50 cc. of 2 per cent $NaHCO_3$ with 1 gram of suspended $CaCO_3$, the rate of flow was not affected.

Then an animal was prepared in the usual manner with a fistula and in addition provision was made for the reintroduction of the pancreatic juice into the duodenum a few inches below the ampulla. After secretion rate had been observed for a while, the juice was returned to the intestine from a drip apparatus in large quantities with the following results.

In two experiments 100 and 250 cc. of juice respectively were introduced, at a rate between 0.5 and 1.5 cc. per minute. The mean rate of flow from the pancreas was observed before, during, and after this procedure and was found to be undiminished. The figures were 0.120, 0.160, and 0.150 cc. per minute respectively. A similar experiment was performed, introducing the juice through a tube by mouth into the stomach rather than through an opening in the duodenum. In this dog the original rate varied between 0.253 and 0.155 cc. per minute. While the juice was being introduced the rates varied between 0.200 and 0.392 cc. per minute. Again there was no inhibition of flow.

Finally, an animal was prepared with a system of altercursive intubation as described by Elman and McCaughan (11), by means of which the pancreatic juice was continuously returned to the intestine through the biliary tract. Briefly, this consists of an intercommunicating system of

tubes leading from the pancreas to the gall bladder, arranged in such a way that the flow of fluid can be observed outside the body. By this procedure the pancreatic juice was allowed to enter the duodenum through the ampulla and neutralization of gastric HCl could continue as in the normal animal. Elman (24) has shown that the gastric hypersecretion which he reports as accompanying complete drainage to the outside does not occur under these conditions.

For the first three days after intubation, pancreatic juice was collected separately in a rubber bag, in order to determine whether the juice flowed freely. On the third day, 340 cc. of juice were collected in 24 hours. The bag was then removed and the juice allowed to flow into the intestine

TABLE 2

Dog 98. Altercursive intubation

Rate of pancreatic secretion in fasting animal after juice was continuously returned to intestine for 7 days.

DATE	LENGTH OF PERIOD	RATE PER MINUTE	REMARKS
	minutes	*cc.*	
	10	0.240	Dog fasting 24
Nov. 30	10	0.175	hours
	10	0.130	
	10	0.140	Dog fasting 56
	10	0.215	hours
	10	0.150	
	10	0.200	
	10	0.125	
Dec. 1	10	0.115	
	10	0.125	
	10	0.095	
	10	0.115	
	10	0.140	

through the biliary system. On the following day examination of the T-tubes revealed that they were both filled with clear pancreatic juice and no bile was visible. The outlet clamp upon the pancreatic side of the system was then released momentarily until a column of bile appeared in the tube on the biliary side of the system. The clamp was then re-applied and in about 20 minutes the bile had disappeared and was replaced by pancreatic juice. This preliminary observation indicated that the tube system was clear and that pancreatic juice was flowing through the biliary system.

Seven days later, the animal was deprived of food for 24 hours, water being allowed freely. During this time the juice had been made to flow

into the intestine. After the 24 hour fast, observations were made on the rate of pancreatic secretion. Then fasting was continued for the next 23 hours, water being given freely, during which time the juice again was allowed to flow into the intestine. The secretory rates are shown in table 2.

In order to avoid the possible criticism that the lack of alkaline pancreatic juice in the intestine during the collection periods set up an immediate secretion due to secretin production, the experiment was modified so

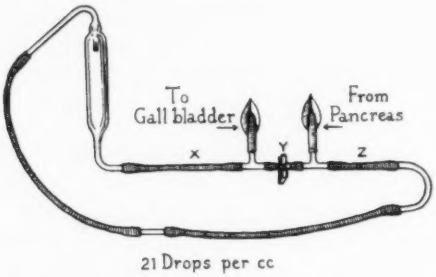

Fig. 2. Arrangement of drop counting bulb altercursive intubation

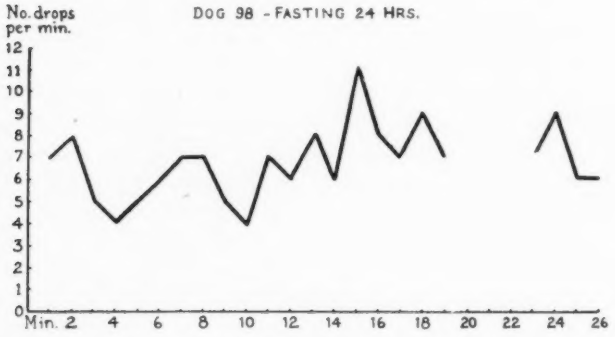

Fig. 3. Continuous secretion of pancreatic juice continuously returned to intestine.

that the return of juice to the intestine was never interrupted. A glass bulb with a glass tip leading into the top of it from the pancreas side and an outlet tube from the bottom of the bulb leading to the bile side of the altercursive system was introduced as shown in diagram (see fig. 2). This should give no possible opportunity for unneutralized HCl forming the postulated extra secretin and still the pancreatic flow continued. The results are shown in figure 3.

In order to show that the juice was flowing in this artificial system and

was being returned to the intestine during the intervals between observations, the pressure and resistance factors were studied. A T-tube manometer was inserted between the two duct systems in place of the glass bulb. When the clamp at *X* was applied with *Y* closed, the column of fluid gradually rose to a height of about 270 mm. (see B, fig. 4). This indicated the apparent pancreatic secretory pressure and corroborated our previous findings. The clamp at *X* was then removed and the column of fluid dropped abruptly, fluctuating between 160 and 110 mm. (see C to D, fig. 4). The sudden drop was due to the free entrance of juice into the

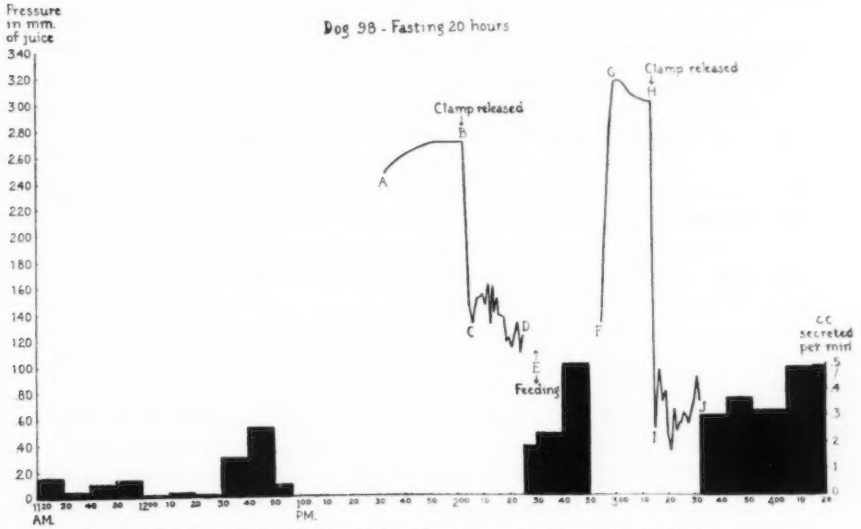

Fig. 4. Altercursive intubation (juice having been returned to intestine continuously for 11 days). Pancreatic secretory pressure and resistance—before and after food.

Lines in upper part of chart are pressure data with scale on left hand side. Blocks in lower part of chart give secretion rate with scale at right.

intestine and since the resistance at the sphincter of Oddi as determined by Elman and McMaster (25) varies between 100 and 120 mm. the column of fluid remained at that level temporarily. In another experiment done after 56 hours of fasting the column of fluid in the manometer rose to a height of 310 mm. and on opening of clamp, it fell promptly and fluctuated between 95 and 115 mm. The secretory pressure was always sufficient to insure entrance of juice into the intestine.

We are well aware that the conditions under which we made these observations differ somewhat from those of McMaster and Elman. Their

measurements refer to a biliary system in which the gall bladder had been removed, while in our animals the gall bladder was present and carried a cannula. But since our figures agree very well with those of McMaster and Elman, we feel that they may stand as they are. Further investigation along the ingenious and fruitful lines suggested by the two papers of McMaster and Elman (22, 25) would be very desirable. At this point it seems worth while to emphasize that in the discharge of secretion into the intestine the important point with regard to the pancreas is the continuous high pressure within the ducts, while in bile secretion the lowering of resistance to the outflow (22, 25) is a decisive factor.

Twenty-five minutes after giving one-half pound of raw meat the manometer was again attached and the pressure rose to 316 mm. (see F to G, fig. 4). When the clamp was released pressure fell to a level (I to J) lower than the previous level (C to D). This indicates a lowered resistance at the terminal portion of the common duct after feeding and agrees with findings reported by Elman and McMaster (25). The fluctuations in manometer readings after flow of juice into the gall bladder probably represent variable muscular tone in the intestinal wall. Elasticity of gall-bladder wall may also play a part. The fluctuations are not respiratory since all readings were taken as mean points of the respiratory movement which can be quite easily distinguished.

Serum amylase determinations showed that the flow of juice was not interrupted. Previous experiments have shown that when any obstruction occurs there is a distinct rise in blood amylase.

Summarizing this experiment, we may say that the flow of juice remained continuous in a fasting animal when provision was made for the continuous return of the fluid at its normal site of entrance into the intestine. This shows clearly that the phenomenon of continuous secretion is not the result of a constant secretin effect due to the gastric hypersecretion of HCl and the absence of neutralization of HCl by alkaline pancreatic juice. We should also note that when food is given to a continuously secreting dog, both rate and "secretion pressure" are increased. It is not very plausible therefore to assume that a "*pathological* hypersecretion" is subject to further increase by *normal* food stimulus.

Although in the dog it is practically impossible to measure the resistance at the end of the major pancreatic duct, due to its shortness, we may assume that the resistance is of the same order of magnitude as that at the sphincter of Oddi. It will be noted that when the secretory pressure reached its maximum and then, by releasing the clamp the pancreatic juice was allowed to enter the intestine, a column of fluid measuring from 100 to 150 mm. in height remained in the manometer. This column represented the pressure necessary to overcome the resistance at the terminal portion of the common duct and corresponded to the earlier observations made by

Elman and McMaster (22). The fact that the column of fluid fluctuated at this level indicated that there was a constant flow of juice secreted at a pressure always sufficient to overcome the resistance at the sphincter. Moreover, throughout the entire period of observation there was never any reflux of bile towards the pancreas as long as the system remained closed, showing that pressure in the pancreatic duct was always higher than that within the biliary tract. From this evidence we must conclude that there is no anatomical ground for the hindrance of continuous pancreatic secretion. It is also apparent that in animals which have gall bladders the normal flow of bile into the intestine can go on intermittently while through a common ampulla the secretion of pancreatic juice is continuous. Furthermore, we must concede that a continuous flow of pancreatic juice at a higher pressure than that in the biliary tract will effectually prevent a reflux of bile into the pancreatic duct as long as the pancreas is functioning normally.

Factors concerned in intermittent secretion described by previous investigators. When the methods of Claude Bernard, Heidenhain or Pawlow are used to create a pancreatic fistula, juice is obtained intermittently. During the inter-digestive period secretion is usually not observed and following the ingestion of a meal copious secretion is obtained. With these methods the minor duct is left intact. Under such conditions it is difficult to determine the quantity of juice which enters the intestine through the minor duct, especially in the fasting animal. Moreover, compensatory dilatation of the minor duct may occur during the interval required for healing of the transplanted major duct, at the site of repair. This must remain conjectural, because autopsy reports of the condition of the pancreas and its ducts were not made by those who employed this technique. However, during the inter-digestive phase, most of the secretion may escape into the intestine through the minor duct. During digestion or after the injection of secretin, the secretory pressure and rate of flow are increased and with the introduction of a cannula into the orfice of the fistula, part of the fluid escapes to the outside and part to the intestine. Accurate measurements of the total output are impossible, since the distribution of the juice between the intestine and the exterior depends upon the relative resistance in the two paths (see Babkin, p. 459), the ducts communicating within the gland. It was for this reason that dogs with Pawlow fistulas lived for long periods of time and remained in good condition whereas animals with *complete* fistulas succumb usually after one to three weeks. However, even when the latter dogs are moribund pancreatic secretion continues.

In the experiments of Bayliss and Starling (26), other factors were concerned in creating the impression that they were dealing with an intermittent secretion. Their observations were made in animals under anes-

thesia (A. C. E. mixture). Pancreatic secretion was obtained only after the intravenous injection of secretin. According to our observations this is due to several factors. We have observed repeatedly that the mere introduction of a cannula even with the gentlest manipulation causes a copious secretion of mucus which forms a plug in the duct or cannula and tends to obstruct the flow. Also, ether anesthesia inhibits the secretion of pancreatic juice. This is discussed in detail below. The influence of mucus formation upon pancreatic secretion was demonstrated in our experiments by the fact that relatively small quantities of fluid containing large amounts of mucus were secreted the first two days after operation and not until the third day was the regular rate of secretion attained and did the composition of the juice become normal.

Effect of ether on continuous secretion. One of the striking differences between our experiments and the acute type of experiment in which no spontaneous secretion is found is the use of an anesthetic. The indications in the literature that the pancreas is not indifferent to anesthetics led us to determine whether a dog secreting continuously would be influenced by being subjected to ether anesthesia. Ether anesthesia must, of course, be carefully distinguished from local application of ether in the small intestine. Katsch (28) and others use the stimulating action of ether locally applied to a test for external secretory function. Many other substances have a similar action. These agents probably work through the secretin mechanism.

The observations recorded in table 3 show that ether has a marked inhibitory effect upon the rate of pancreatic secretion. The effect manifested itself within the first 10 to 15 minutes after the induction of the anesthesia and lasted 7 hours. This is the typical result. It is well known that some dogs have a greater susceptibility to ether. In these only a light anesthesia can be maintained and the effect on the pancreas is less marked.

In this connection it is of interest to note that an animal can be maintained under a light surgical anesthesia with sodium amytal with only a slight inhibition of flow of pancreatic juice (see table 4).

A single injection of amytal in a dose of 25 mgm. per kilo was sufficient to induce surgical anesthesia and the rate of flow of juice was slightly slowed. When the injections were repeated more marked inhibitory effects occurred. A number of times when a normal dog which had fasted for 24 hours, was anesthetized with amytal, it was evident when the duodenum was opened opposite the papilla that spontaneous secretion was going on. With avertin anesthesia this was also found. If the surface was kept well moistened, secretion could be observed for quite a while. Slight irritation or the insertion of a cannula into the duct caused an outpouring

of mucus which quickly clogged the cannula and prevented the further flow of juice *at fasting secretory pressure.*

Effect of ether on serum amylase. So far we have shown that in a dog whose pancreas is cannulated by the Rous-McMaster technique the secretion is continuous. There is reason to believe that the condition of such an animal is more nearly normal than that of animals under anesthesia.

TABLE 3

Dog 87. Effect of ether anesthesia on continuous secretion of cannulated pancreas

	LENGTH OF PERIOD	AMOUNT SECRETED	RATE PER MINUTE	SERUM AMYLASE
		cc.	*cc.*	
1st preliminary period	9	4.0	0.444	80
2nd preliminary period	14	4.55	0.325	
3rd preliminary period	13½	4.10	0.300	
4th preliminary period	17	4.35	0.256	
Mean rate per minute for all 4 periods			0.331	
2:20 p.m. During induction of anesthesia	15	1.65	0.11	
2:35 p.m. Under anesthesia lasting 1½ hrs.	14	0.65	0.05	
2:50 p.m.	9	0.15	0.016	
3:00 p.m.	7	0.00	0	
3:08 p.m.	6	0.05	0.009*	
3:14 p.m.	34	0.1	0.003*	100
3:47 p.m.	48	0.1	0.0017*	100
4:37 p.m. Recovered from anesthesia 2 hrs. after induction	83	0	0	
6:00 p.m.	60	3.5	0.058†	
7:00 p.m.	75	4.0	0.053†	
8:23 p.m.	50	1.7	0.035†	
9:15 p.m.	10	2.8	0.28	
9:25 p.m. 7½ hrs. after induction of anesthesia	11	4.75	0.432	160

The various periods in the table were so chosen as to indicate changing rate of flow. The * indicates that the period contained several 0 per minute readings; † indicates that collection was made in rubber bag instead of pipette.

Before we conclude, however, that continuous secretion is a normal process in the dog similar to the continuous secretion of rabbits and of ruminants, we require some independent evidence that pancreatic activity is continuous in the unoperated animal. This information was obtained by studying the serum amylase of dogs subjected to ether anesthesia as reported in the following paper. Since it is generally conceded that no other

TABLE 4

Dog 89. Effect of amytal on continuous secretion of cannulated pancreas

Sodium amytal (Lilly) given intravenously

	LENGTH OF PERIOD	AMOUNT SECRETED	RATE PER MINUTE
	minutes	*cc.*	*cc.*
1st preliminary period	10	1.18	0.118
2nd preliminary period	10	1.67	0.167
3rd preliminary period	13	2.3	0.177
0.4 gram amytal	10	0.4	0.04
	10	1.0	0.10
	10	1.3	0.13
	9	1.45	0.161
	13	1.5	0.115
	10	1.10	0.110
0.3 gram amytal	10	0.9	0.09
	11	0.5	0.045
	10	0.63	0.063
	10	0.35	0.035
	10	0.85	0.085
	10	0.61	0.061
	10	0.40	0.040
	7	0.54	0.054
	10	1.32	0.132
	10	1.10	0.110
	10	1.15	0.115
	10	0.93	0.093
0.2 gram amytal	10	0.54	0.054
	10	0.33	0.033
	10	0.28	0.028
	7	0.22	0.022*
	13	0.25	0.019
	15	0.33	0.022
	15	0.27	0.018
	13	0.30	0.023
	17	0.15	0.009*
	17	0.95	0.056
	11	0.35	0.032
	9	0.28	0.031
	15	0.42	0.028
	10	0.30	0.030
	25	0.70	0.028
	26	0.61	0.023
	hours		
	16	70.	0.073

* Indicates periods containing several minutes with 0 cc. per minute reading.

procedure, except interference with the escape of juice from the gland will produce such effects, we can safely conclude that the influence of ether anesthesia on blood amylase is due to inhibition of excretion from the gland, while the continuous tendency to form juice is not impaired.

SUMMARY

1. In dogs whose pancreas is cannulated according to the Rous-McMaster technique secretion is observed to be continuous. Food or the injection of secretin temporarily increases the rate of flow. Secretin juice is secreted under a higher pressure than that of continuous secretion.

2. Anesthesia may completely inhibit the flow of pancreatic juice. With ether the effect seems to be more marked than with amytal.

3. Ether anesthesia increases the amylase level in the blood. This is explained on the basis of the double mechanism of formation and transport of pancreatic secretion. Ether abolishes the latter, thereby raising the blood amylase level through resorption of the enzyme.

4. In dogs with "altercursive fistulas" where there is admittedly no gastric hypersecretion and no lack of neutralization of gastric HCl, the spontaneous secretion can be demonstrated after 48 hours of fasting. The conclusion seems inevitable that continuous pancreatic secretion is a normal phenomenon and not "pathological hypersecretion."

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THE AMYLASE OF SERUM IN RELATION TO FUNCTIONAL STATES OF THE PANCREAS

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The literature on blood amylase has been very completely reviewed by Oppenheimer (1). With regard to serum amylase as an indication of the functional state of the pancreas there is good general agreement on many of the main features, certain rather important points, however, are still in dispute. It is conceded that the serum amylase is principally derived from the pancreas and that ligation of the ducts promptly leads to an increase. In carnivora all other organs are so much lower in amylase content than the serum, that any large increases are probably derived from the pancreas. We confine ourselves in this paper to conditions in the dog where salivary amylase is negligible. It is important to note (Anrep and others (2, 3, 4)) that pancreatic secretion comprises two mechanisms under separate control, the formation of juice and its transfer to the intestine. The enzyme enters the blood stream either directly from the gland or via the lymph channels. When the outflow is blocked, the serum amylase rises.

Some of the points which are not yet sufficiently clarified involve questions regarding the fate of serum amylase (excretion and possible destruction), reabsorption from the intestine and the effect on serum amylase of certain drugs which apparently affect pancreatic function.

METHODS. Serum amylase was determined by the Wohlgemuth (5) methods at pH 6.2 in the presence of a constant concentration of NaCl, as suggested by Michaelis (6). Over a range of 0.1 cc. to 5.0 cc. the action of a 1:100 dilution of a serum was determined in a reaction mixture of 10 cc., whose final starch concentration was 0.02 per cent. A stock solution containing 0.4 per cent soluble starch and 0.6 per cent NaCl will keep in a refrigerator for about a month unchanged. We make fresh solutions about every two weeks. For use this is diluted with an equal volume of M/20 phosphate buffer. Digestions are done at 37.5° for 30 minutes. The amylase of pancreatic juice and urine are determined similarly. A method of this kind, besides lacking in precision is also quite subjective when it comes to matching shades of colors in tubes. With some experi-

ence and careful repetition of tests at different dilutions when the first series does not show good demarcation, we believe that the results are within an error of about ± 5 per cent to 10 per cent. With the large differences usually recorded the method is satisfactory enough.

The figures recorded in this paper are calculated from the enzyme dilutions as conveniently used and are not intended necessarily to represent accuracy to the last digits. We use Wohlgemuth's designation D for amylase units.

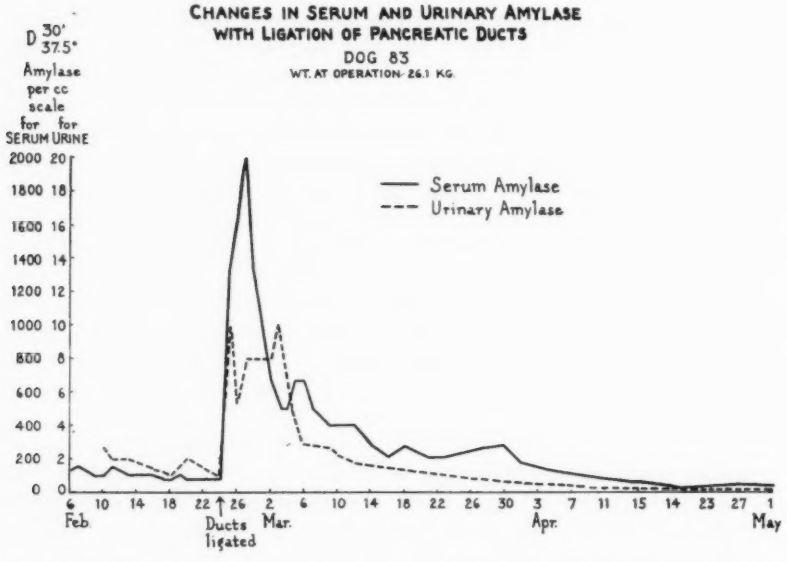
For certain purposes lipase determinations have been included in this paper. Methods for serum lipase have been discussed by Cherry and

CHART I

CHANGES IN SERUM AND URINARY AMYLASE WITH LIGATION OF PANCREATIC DUCTS

DOG 83

WT. AT OPERATION- 26.1 KG.



Crandall (7). In the experiments recorded here, in order to compare results with those of Crandall and Cherry, the determinations were carried out essentially as described by them. It might be preferable to observe the precautions laid down by Willstätter (8).

SERUM AMYLASE AND URINARY AMYLASE AS INFLUENCED BY LIGATION OF THE DUCTS, PANCREATECTOMY AND FISTULA. *Ligation.* It is well established that ligation of the ducts increases the amylase in the serum and also in the urine. The curves (chart I) for serum and urinary amylase rise and fall in a rather parallel manner. An attempt to correlate quantitatively the level of amylase in serum and excretion in urine shows that the amylase lost to the serum after the peak is reached cannot by any

means be accounted for in the urine. It is perfectly apparent that the drop of hundreds of units per cubic centimeter in the blood with a blood volume of 1,500 to 2,000 cc. is not reflected in the urine where the largest change from day to day does not exceed 10 units.

We have often been surprised during the last few years by the stability of the amylase of pancreatic juice and serum kept in the refrigerator. The values will remain constant within the limit of error of the method for weeks particularly in the sterile pancreatic juice. In urine, however, the conditions are not at all the same, very noticeable deterioration occurring in a few hours at room temperature. Pancreatic juice diluted with dog urine 1:1000 gave the expected value if the determination was done immediately but showed a 30 per cent deterioration in 21 hours while a similar dilution of 1:10,000 showed more than 50 per cent loss. Corresponding dilutions with distilled water showed a 60 per cent and 80 per cent loss of enzyme activity.

When tested for deterioration of amylase in shorter periods, urine showed in three samples of 50 to 75 cc. of catheterized urine, representing 2 to 3 hours' renal activity, a nearly immediately noticeable deterioration with 30 per cent to 40 per cent destruction in 6 hours. After that the rate of disappearance became notably slower. Apparently the first few hours while in the bladder at body temperature may have an effect which makes determinations on 24 hour samples entirely misleading, involving an error approaching 50 per cent. However, even allowing for a 90 per cent deterioration of amylase in the 24 hour sample, we cannot account for the rapid changes in serum amylase by excretion through the kidney. Logarithmic extrapolation for zero time of the maximal slopes for deterioration would not raise the values to double the recorded figures. It is very probable that the urine collected for 24 hours can no longer be relied upon to show correct values which would represent the actual amount of amylase which has passed out of the blood in the kidney. A part of the deficit of amylase in the urine is therefore explained by deterioration when diluted in the urine. By far the greater part, however, must be accounted for otherwise.

Another mechanism of dealing with high serum enzymes is of considerable interest. This is fully discussed in the early editions of Oppenheimer's Handbuch der Biochemie (9, 10). As long ago as 1879, Langendorff (11) suggested that in birds with ligated pancreatic ducts, when the serum enzymes rise, they reach the intestine by some other than the direct route. Abelman (12) and Rosenberg (13) came to the same conclusion. Schegalow (14) determined increased proteolytic activity in the bile after ligation of pancreatic ducts and Lombroso (15) found the same for lipase. Lombroso's observations were verified by Pflüger (16). Our own data indicate that this situation also holds for amylase. In a dog with functioning bile

fistula, the pancreatic ducts were ligated. A comparison of the amylase as well as lipase values before and after the ligation showed that the two enzymes in the bile rose from nearly negligible amounts to a very considerable concentration. This we think is a very important fact and is now the subject of further investigation. It appeals to us as a very ingenious mechanism of returning the pancreatic enzymes to the intestine when temporary occlusion of the ducts occurs. Under these conditions the opinion of Carlson (17) that serum amylase is simply a waste product on the way to excretion has to be somewhat modified. It also offers a satisfactory explanation for the observations made by many observers since the beginning of experimental studies on the pancreas, that the effect on digestion in the intestine without pancreatic juice differs when the pancreas is removed or merely ligated. Cruikshank (18) in a careful study showed that the enzyme digestions in the intestine are really very little interfered with by ligation of the pancreatic ducts while a more serious disturbance ensues when later the whole gland is removed. How much of the digestive load is carried by the enzymes secreted normally by the intestinal mucosa and how much of the disturbance in digestion after pancreatectomy is referable to loss of internal secretion cannot be stated at the present time.

Crandall and Cherry (19) suggest that there is normally a destruction of lipase and to a lesser extent of amylase in the liver. When secondary liver changes set in, due to interference with pancreatic function, the enzyme destroying power of the liver is decreased. A rise in blood lipase then occurs. We do not believe that the rapid fall in blood amylase which we find after the peak has been reached can be explained by any change in rate of enzyme destruction in the liver. Besides, according to Crandall, at the time when the liver changes begin to set in, the enzyme should rise, while as a matter of fact it falls. This subject will be dealt with in more detail further on. Although enzyme destruction in the liver will not explain the drop of amylase after the peak, excretion by the liver may account for it. Curious results are sometimes obtained after ligation of the ducts. It is absolutely necessary to do careful autopsies in all cases. We have encountered both the phenomenon of atrophy—regeneration—reatrophy fully discussed by Bensley (20) and also the establishment of recommunication with the intestine described first by Pawlow (21) in rabbits and by Pratt (22) and co-workers in dogs.

Fistula. When a pancreatic fistula is made the blood and urinary amylase values run a course very similar to that after ligation. This is easily understood because during the first few days after a fistula operation there is virtually a complete obstruction of the ducts. We prefer to wait for the juice to flow spontaneously and do not feed the dogs before operation as Elman does to obtain prompt secretion. The vomiting Elman

records as a constant part of the picture is apparently due to this feeding. The fall after the peak cannot be explained by the establishment of flow because the flow, as seen in chart II, may set in after the beginning of the fall. The mechanism must be similar to that after ligation in which excretion in the bile probably is a large factor. Elman and McCaughan (23) report unchanged values when the blood was taken "several days" after beginning of flow. The initial rise in our curve is to be explained by temporary obstruction and will vary from animal to animal. That given in the chart represents the average result. It begins with the inception of anesthesia. The fall below normal may be due either to lessened resistance to outflow or to functional atrophy. After long-standing fistulas the pan-

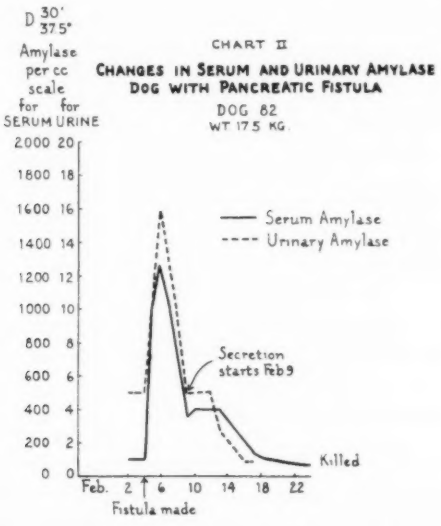


CHART II

CHANGES IN SERUM AND URINARY AMYLASE DOG WITH PANCREATIC FISTULA

DOG 82
WT 17.5 KG.

creas shows a decreased volume and appears grossly somewhat shrunken without, however, any microscopic cellular changes.

Pancreatectomy. In pancreatectomy a lowering of the serum amylase is recorded by practically all observers but the interpretations of the data vary a good deal. The results are somewhat dependent on the method used. Some of the observations have been made on glycogen instead of starch. There are many indications that the pancreatic polyase acts more specifically on starch, while glycogen is more subject to action of polyases from other tissues. There is some divergence in the results obtained with starch and with glycogen and "a return toward the normal diastase values" after pancreatectomy has been noted only when glycogen was used (24). Willstätter (15) and others also hold that pancreatic amylase hydrolyzes

starch down to maltose only. Serum, however, produces glucose among the starch hydrolysis products. This is interpreted as due to the presence in the serum of maltase which is absent from the pancreas. The presence of maltase, besides leading to divergent results with the various methods for determining extent of hydrolysis, certainly also has an effect through displacement of the equilibrium no matter what method is used. When we try to answer questions regarding the relation of the pancreas to serum amylase all of the above considerations are pertinent. More than elsewhere this is true after removal of the pancreas when we are dealing with the much reduced amylolytic activity of the serum. A résumé of the results on six pancreatectomies is given in table 1. In the first 24 hours there is a drop to somewhat less than half the original value. After this there is no further decided change, the fluctuations being within the error of the method. In a few experiments with glycogen as substrate there

TABLE 1

Pancreas extirpation and serum amylase

Serum amylase $D\,^{30'}_{37.5°}$

DOG NUMBER	INITIAL VALUE	DAYS AFTER OPERATION						
		1	2	4	7	18	21	31
86	66.6	26.6	20.0	26.6	26.6			
105	57.2	22.2		22.2				
112	50.0		25.0					
113	80.0	36.3		28.6				
114	66.6		30.8					
118	89.0			40.0		44.5	36.4	35.0

appeared to be a very much smaller initial drop and with some fluctuation a definite tendency to return toward the normal. This coincides with the findings of Milne and Peters (24) and in our experience occurs only when glycogen is used. The starch hydrolysis values never showed a secondary rise even after 31 days under insulin treatment but neither does the serum amylase ever disappear entirely. Very small amounts also continue to appear in the urine. The simplest interpretation would be that a little more than half of the serum amylase comes from the pancreas and the rest has its origin in other organs. We are not certain, however, that this is the only explanation possible. Disregarding for the present the question of strict specificity of the polyases, other tissues may store pancreatic amylase and gradually give it up again when the level in the blood is low. We are also giving further attention to the possibility that the circulating amylase is more slowly excreted the lower the level in the serum. As we have pointed out, although the determination of amylase excreted by the

kidney is very unsatisfactory, the amounts excreted in the urine are very small compared with the concentration in the blood. The threshold for excretion into the bile is apparently higher than that for the kidney since urine always contains quantities at least within the range of ordinary methods while normal bile has hardly more than traces. A good deal is to be said for this point of view which leaves open the possibility that (in the dog, at least) the origin of amylase is exclusively to be sought in the pancreas. Final disposition of this question must be deferred until we know whether or not the amylase of the pancreas and the glycogenase occurring in the pancreas and elsewhere are specific enzymes in the strictest sense.

Is amylase reabsorbed from the intestine? Oppenheimer's (1) review states that amylase is reabsorbed, quoting as the only evidence the old experiments of Wasserthal (26), in which excised intestine was suspended as a dialyzing tube containing saliva and after 24 hours amylase was detected in the outside fluid. After 48 hours there was more. This, of course, proves nothing regarding conditions in the living animal. So far we have found no further evidence for reabsorption of amylase in the literature, but in a great many places the tacit assumption that it is reabsorbed. On the other side of the argument we have the well controlled observations of Moeckel and Rost (27) that when pig's serum, which is ten times as high in amylase as dog's serum, is fed to a dog, no increased amylase can be detected in the dog's serum. A number of experiments were performed on animals under conditions more favorable than those of Moeckel and Rost. Juice was introduced either into the stomach or by duodenal sound into the duodenum, or through an incision directly into the duodenum. There was never any sign of absorption of amylase. The serum samples up to 8 hours afterwards did not vary by one tube in the Wohlgemuth test. As an example we give the following: dog 89: July 15, 1931; juice from dog 89 of July 14 used containing 4,000 units per cc. Two hundred fifty cubic centimeters introduced at a rate varying from 0.5 to 1.5 cc. per minute. Infusion occupied 4 hours. Methylene blue added to the juice was absorbed normally. The initial value of serum amylase was 40 and after $1\frac{1}{2}$, 3, $5\frac{1}{2}$, 7 and 8 hours, the value had not changed. The dilutions used indicated that the assigned value of 40 units was not higher than 45 and not lower than 36. This experiment was by direct infusion into the duodenum, the animal having fully recovered from the operation for tube insertion.

On general grounds we believe it highly improbable that enzymes should be absorbed. There is pretty general agreement that enzymes involve a protein-like structure (on composition of amylase, see Sherman and Gettler, 28) and aggregates of this kind would probably not be absorbed in their original form. If enzymes are generally capable of passing the intestinal mucosa unchanged, it ought to be very easily demonstrable with

amylase from pancreatic juice which has at least 50 times the concentration that occurs in serum. A rather convincing case against absorption is also presented by urease. Evidence of urease action within the body is easily obtained when it is introduced parenterally while urease taken by mouth shows no such results. (See Oppenheimer (1).) In fact, animal life would seem to be a much more precarious thing than it really is if enzymes carried in raw food materials could freely pass the intestinal barrier.

THE EFFECT ON SERUM AMYLASE OF DRUGS WHICH AFFECT PANCREATIC FUNCTION. *Ether.* Elsewhere (29) we have shown that the continuous secretion of a pancreas cannulated by the Rous-McMaster technique is inhibited by ether anesthesia. In the fistula animals we noticed a variable susceptibility to ether anesthesia, and also a variable duration of inhibition of flow. In studying the serum amylase, therefore, we made sure of having a sufficiently long anesthesia period and also a long period of observation after recovery from ether. We found that as a rule it takes two hours to show the first effects. This is not always certain, being too near the limits of the method and we interpret the two hour effect only when there is a continued increase found in the later observations. The peak usually comes at 7 to 9 hours.

Carlson and Luckhardt (17) and Davis and Ross (30) had made their observations on the effect of ether at maximally two hours, with negative or doubtful results, so that there is no contradiction between their observations and ours. It appears that no matter under what conditions amylase passes from the pancreas into the blood stream about two hours is the minimum time required to show a measurable rise by the methods used. We have never observed an appreciable change in 15 minutes as Elman (31) reports on the basis of a viscosimetric method.

We interpret this rise as being due to an unequal action on the two mechanisms (Anrep) concerned with pancreatic secretion. Ether anesthesia causes an inhibition of the mechanism of juice transfer while the juice (and enzyme) producing mechanism is left unaltered.

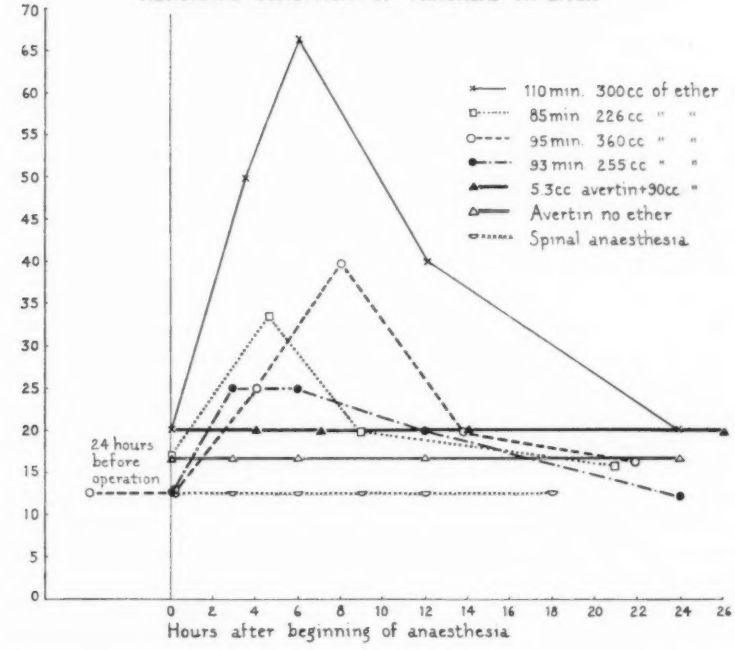
There is a close parallelism between mechanical stoppage or clamping of the tube and the ether anesthesia effect. This is shown by the rate at which the serum amylase rises with these various procedures in fasting animals. In four dogs under ether anesthesia the amylase rose at the rate of 20 to 25 units when calculated per hour up to the time of the peak; clamping the rubber tube in a fistula animal resulted in an hourly increment of 20 units, while accidental stoppage (kinks or mucous plugs) gave a rate of 20 to 30 units. A higher rate, of about 50 units per hour, was found only immediately after the operation of ligating the ducts which involves also handling of the gland itself and may certainly be expected to lead to more profound disturbances.

These data leave very little doubt that the effect of ether on blood amylase comes about through an interference with that part of pancreatic function which has to do with the transfer of juice from the cells to the intestine.

In a number of human surgical patients amylase studies after various forms of anesthesia were carried out. The results agree with those on dogs and are given in chart III. Ether produces a marked rise while avertin and spinal anesthesia have no effect.

CHART III

SERUM AMYLASE AFTER ETHER ANAESTHESIA IN HUMAN SURGICAL CASES WITH NO KNOWN ABNORMAL CONDITION OF PANCREAS OR LIVER



Chloroform. Before discussing the effect of chloroform poisoning a few words may be in place on the rôle of secondary changes in the liver influencing serum enzymes. Crandall and Cherry (19) claim that in pancreatic lesions or ligation of the pancreatic ducts it is not the condition of this organ that influences serum enzymes, but that the secondary changes in the liver control enzyme level in the blood on the basis of an alleged enzyme destroying power of the liver. We believe with Langendorff, Rosenberg,

Abelmann, Shegalow, Lombroso and Pflüger and from the data in the latest paper of Cherry and Crandall (32) that when the pancreatic function is acutely interfered with and a prompt rise in serum enzymes occurs it is not necessary to invoke secondary changes in the liver which are delayed in their appearance, to explain a rapid rise in serum enzymes. It is certainly true that by the time the marked rise in serum amylase has occurred after ligation of the pancreatic ducts, the liver still shows a perfectly normal appearance in section. The visible changes set in only after 2 to 3 weeks (33). On the other hand, when primary changes occur in the liver such as in chloroform poisoning a marked outpouring of lipase from the injured or destroyed cells into the blood stream has been recorded (34), (35); Crandall and Cherry claim that they can demonstrate the normal

TABLE 2

Serum amylase and lipase after liver injury

By injection of 1.0 cc. chloroform per kgm.

CONDITION	CAT 719		CAT 720		CAT 722		CAT 723	
	Amylase $D^{30'}_{37.5°}$	Lipase as cc. N/50 NaOH	Amylase $D^{30'}_{37.5°}$	Lipase as cc. N/50 NaOH	Amylase $D^{30'}_{37.5°}$	Lipase as cc. N/50 NaOH	Amylase $D^{30'}_{37.5°}$	Lipase as cc. N/50 NaOH
Normal	57.2	0.45	40.0	0.40	57.2	0.10	50.0	0.05
After $CHCl_3$								
1 day					28.6	0.65	44.5	0.45
2 days	40.0	0.80	22.8	1.00	25.0	1.10	40.0	1.50
4 days					36.3	1.10	44.5	1.40
7 days					50.0	0.80	50.0	0.40
14 days					Dead		50.0	0.30

enzyme destruction in the liver by shunting the portal blood supply past the liver by means of an Eck fistula which results in a raised serum lipase. It is true that the immediate effect of an Eck fistula is largely only a circulatory shunt, but after some time marked degenerative changes set in. Crandall and Cherry's increase in serum lipase is also delayed. We would hesitate to compare the later results of an Eck fistula with the secondary liver changes resulting from interference with pancreatic function as far as effect on serum enzymes is concerned. In the Eck fistula the liver changes are primary. If in this condition the serum enzymes rise at a time when the liver volume has shrunken and marked changes have set in the result is probably quite parallel to chloroform poisoning in the mechanism which produces it. The time of onset is quicker in the more acute chloroform effect.

In contrast to all the above, we wish to show that there is also a direct

effect of chloroform on the pancreas besides its action on the liver. This cannot be confused with the lipase-liver phenomenon because the direction of its effect is opposite.

The effect of chloroform in lowering serum amylase has been previously described by Davis and Ross (30) who further state that this does not occur in animals with extirpated pancreas. What we wish to emphasize particularly at this point is that the effect of chloroform on amylase is through the pancreas and is to be carefully distinguished from the effect on lipase which occurs by an entirely different mechanism involving the liver.

The level of serum amylase can be influenced by a number of factors involving the pancreas. No marked change in serum amylase level has been produced in which the pancreas does not play a dominant rôle. We are inclined to refer all these changes definitely to effects on one of the two mechanisms involved in the external secretory process. Whenever outflow or transfer to the intestine is interfered with the amylase rises. This is brought about by mechanical effects, including trauma or by ether anesthesia. It would be interesting to know whether any other drugs produce similar effects. When there is a lowered elaboration of juice (and enzymes) the serum amylase is lowered. Decreased resistance also seems to have this effect. Lowered serum amylase is brought about by fistula by complete removal of the gland (in partial removal trauma may raise it), by atrophy and by chloroform. When the resistance to outflow is normal secretin has no effect on serum amylase.

COMMENT. When we began our studies on the pancreas a number of years ago, we had the impression that the determination of enzyme activity in serum and in urine was not subject to very much useful interpretation for purposes of pancreas physiology. The many contradictions in the literature discouraged us. As the work progressed, however, we recognized some of the obvious pitfalls which could be avoided. The instability of urinary amylase warned us away from any conclusions based on urine examinations. When we encountered regeneration and reatrophy of the pancreas (20) certain apparent contradictions cleared up. We recognized that in long standing ligations the results can be understood perfectly well, but conclusions must be drawn with great care. The curves for serum amylase plotted against time since operation made it clear that the results of any experimental procedure can be judged correctly only if the time element is considered. The striking effects of ether and of chloroform (observations with other drugs have also been in progress for some time) showed that the picture can be greatly modified by factors which so far have received little consideration. A demonstration of the non-absorption of amylase from the intestine assured us that the serum enzyme situation, in one respect at least, is simpler than had been generally assumed. A clearer knowledge of the relation of spontaneous secretion to the secretin

phenomenon will also further our understanding of the physiology of the pancreas. We believe that on the basis of these and other considerations it is worth while to reinvestigate the whole subject. The progress that has in recent years been made on the chemistry of enzymes warrants the hope that the attempt at biological application of such knowledge may be fruitful. It is our desire in this paper to lay down certain fundamental biological observations which seem necessary for a more intensive study.

SUMMARY. There is little positive evidence that amylase from other organs besides the pancreas can affect the serum amylase. Here it differs from serum lipase which may easily be increased through tissue injury particularly in the liver. Through mechanisms inherent in the pancreas, the serum amylase may be either raised or lowered.

Amylase is not absorbed from the intestine. We believe that, except for excretion into bile or urine, any definite changes in serum amylase level are referable to the pancreas.

The following illustrates the changes so far studied experimentally:

Factors affecting serum amylase

THROUGH THE PANCREAS		NOT THROUGH THE PANCREAS
Raising	Lowering	
Ligation (early stage)	Extirpation	Excretion by kidney
Fistula (early stage)	Fistula (late stage)	Excretion in bile
Ether anesthesia	Ligation (late stage)	
(Trauma)	Chloroform poisoning	

CONCLUSIONS

1. After pancreatectomy the serum amylase falls to less than half the normal value and does not recover. When glycogen is used as substrate the measured effect is less and also inconstant.

2. In animals with ligated pancreatic ducts there is a progressive rise in serum amylase for two or three days. A steep fall to nearly normal values ensues in the next few days after which it slowly sinks to values about half the normal.

3. In fistulas the serum amylase runs a course similar to ligation with, however, a less steep rise and a quicker return to normal.

4. The effect of ether anesthesia is equivalent to a temporary obstruction. This is also shown in human beings.

5. Chloroform lowers the serum amylase with recovery to normal in a few days. It raises the serum lipase (substrate: triglycerides).

6. Amylase is not absorbed from the intestine.

7. Normal liver bile contains only traces of amylase. When the serum amylase rises, excretion in the bile occurs.

8. Amylase is readily excreted in the kidney but there is considerable destruction in the urine. Urine amylase determinations are therefore not very significant.

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FURTHER OBSERVATIONS ON GLOMERULAR FUNCTION

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In an earlier report (White, 1928) the molecular concentration of glomerular fluid in Necturus was stated to be greater than that of serum. The comparisons were made by Barger's technique (1904). Wearn and Richards (1925) had reported a higher concentration of chloride in glomerular fluid than in the serum of frogs. A later paper by Freeman, Livingston and Richards (1930) stated that the percentage of cases in which the chloride content of glomerular fluid was significantly higher than that of the serum in living frogs was lower than in the series of Wearn and Richards. In three other papers simultaneously published from Richards' laboratory it was reported that (Richards and Walker, 1930) the concentration of dye in the glomerular fluid of frogs was the same as in a plasma ultrafiltrate, that (Walker, 1930) the molecular concentration of glomerular fluid in frogs and in Necturus, as compared by Barger's method, is the same as that of the plasma, and that (Bayliss and Walker, 1930) the electrical conductances of the glomerular fluid and of a plasma ultrafiltrate were the same. Instances where the glomerular fluid appeared more concentrated than the plasma were thought to be due to experimental errors, the most important of these being evaporation of the glomerular fluid.

Before the publication of the 1930 series of papers Doctor Richards had informed me of the essential findings. I accordingly began in October of 1929 a second series of experiments with the Barger technique, which are reported in this paper. It is believed that the various opportunities for error, among which are the possibilities of evaporation or diffusion through the capsular wall, leakage around the site of the puncture, damage to the glomerular membrane by the mechanics of the puncture or by interference with the glomerular circulation, drawing up of tubular contents in the frog and Necturus and in Necturus the further possibility of entrance of external fluid through the nephrostome, contamination by the mercury in the pipette and particularly evaporation of glomerular fluid in transferring from collecting pipette to capillary, have been more adequately appreciated and guarded against than in my earlier work. Since the results reported here are essentially a confirmation of results previously reported from Rich-

ards' laboratory, although the experimental procedures have differed somewhat, the details of technique will be omitted.

Apparently the most important defect in my earlier work (1928) was in the technique of transference of fluid from pipette to capillary. The work reported in the present paper consists of 89 experiments on Necturus and 6 on the frog and has extended over a period of 2½ years. Suffice it to say that as the degree of protection against evaporation has increased the percentage of cases in which the molecular concentration of glomerular fluid and of serum were the same has increased. My method for preventing evaporation has been to carry out the transfer of fluid inside a moist chamber containing air saturated with water vapor. In a series of 21 experiments on Necturus performed after satisfactory saturation had been attained the concentration of glomerular fluid was much greater than that of the serum in 2 cases, slightly greater or with doubtful difference in 5, the same in 15 and less in 1. Contamination by tubular fluid was excluded by maintaining positive intracapsular pressure.

In a series of 5 experiments recently carried out in Doctor Richards' laboratory I collected glomerular fluid from Necturus and transferred it to a capillary within a column of paraffin oil. It was then taken over by Doctor Walker and assistants. Their technique for preventing evaporation is to protect the fluid by oil from contact with the air. In these 5 experiments the concentration of fluid was the same as that of the heparinized plasma. In 2 of these cases the concentrations of reducing substances in glomerular fluid and in plasma were determined and found to be essentially the same; this is mentioned as a further indication that the concentration of the fluid had not been changed during or after collection.

In the 6 experiments on the frog I have found the concentrations of glomerular fluid and serum to be the same, although no moist chamber was used. My only explanation of the failure of evaporation to cause trouble here is that smaller tubes, about 400 micra bore, were used in these experiments than with Necturus.

Another source of error with Necturus which has been described separately (White and Lucas, 1932) is the drawing in of fluid from the outside through the nephrostome. Adequate protective measures are now recognized but a failure to appreciate this danger led Schmitt and White (1928) into error regarding the phosphate concentration of glomerular fluid in Necturus. Our finding that the inorganic P content of glomerular fluid was much lower than that of serum was undoubtedly due to our diluting the fluid with phosphate-free salt solution by way of the nephrostome. Walker, Ellinwood and Reisinger found, as they reported at the Philadelphia meeting of the American Society of Biological Chemists, that the inorganic P content of glomerular fluid was the same as that of the plasma in frogs and only a few per cent lower in Necturi. These findings were

communicated to me by Doctor Richards and at his invitation I came to Philadelphia and collected glomerular fluid from Necturi, the inorganic phosphate content of the fluid and plasma being determined by Walker and Ellinwood. In 4 fluids, collected with adequate precautions against the entrance of fluid into the capsule from below, the phosphate content was essentially the same as the average of plasma taken immediately before and immediately after the collection, averaging 6 per cent lower. The figures are seen in table 1.

In two cases, 4/21/32 B and 4/22/32, additional collections of glomerular fluid were made from the same capsules but employing a negative

TABLE 1

DATE	GLOMERULAR FLUID	PLASMA		PER CENT DIFFERENCE
		Average	Before After	
	mgm. P per 100 cc.	*mgm. P per 100 cc.*	*mgm. P per 100 cc.*	
April 20, 1932	5.9	6.2	6.5 5.9	−5
April 20, 1932 B	2.6*	3.0	3.0 3.0	−13
April 21, 1932	3.8	4.1	3.9 4.3	−7
April 21, 1932 B	3.2	3.4	3.2 3.6	−6
April 22, 1932	3.2	3.2	2.9 3.4	0
Average				−6

* Duplicate.

pressure. A second collection from the capsule designated in table 1 as 4/21/32 B but with a pressure such that capsule wall was just collapsed showed 2.6 mgm. P per 100 cc.; a third collection from the same capsule, employing a greater negative pressure, showed only 1.5 mgm. P per 100 cc. while the plasma before and after collection had 3.6 and 3.8 mgm. P per 100 cc. A second collection from capsule 4/22/32 of table 1, employing a negative pressure several millimeters of mercury greater than required just to collapse the capsular wall, showed 1.3 mgm. P per 100 cc. while the plasma had 3.4 mgm. per 100 cc. In these collections where the levelling bulb was deliberately lowered so that a positive intracapsular pressure was no longer maintained, thus reproducing as far as was possible the

conditions of the earlier work of Schmitt and myself, the phosphate content of the glomerular fluid was much less than that of the plasma, while from the same capsules fluid collected under positive intracapsular pressure had shown essentially the same phosphate content as the plasma. This discrepancy is almost certainly due to the drawing in of phosphate-free solution from the outside when positive intracapsular pressure is not maintained, as is shown by the entrance of dye when the levelling bulb is lowered.

A paper (White, 1929) in which it was shown that a glomerulus may continue to eliminate fluid at a time when the sum of intracapsular pressure and measured plasma colloidal osmotic pressure exceeds the measured glomerular capillary pressure may be briefly referred to. This was interpreted as meaning that the glomerulus may continue to eliminate fluid by some process, presumably secretion, at a time when filtration is no longer possible, although under normal circumstances there is an adequate excess of filtering pressure. Since changes in the permeability of the glomerular membrane cannot be excluded in this work it is recognized that one who does not believe in glomerular secretion could, by making not unreasonable assumptions as to the effective colloidal osmotic pressure across the glomerular membrance under the conditions of the experiments, interpret this work as evidence that glomerular function is a pure filtration process.

It is my opinion the best evidence at present available strengthens the view that glomerular function is a passive filtration process. I am convinced that with present knowledge of the existent dangers and the measures available to circumvent them it is possible to collect normal glomerular fluid from the amphibian kidney. The technique of capsular puncture has long since developed beyond the point where such gross errors as touching the glomerular tuft with the pipette tip, uncertainty of the exact position of the tip within the capsular space, undue trauma of the capsular wall or uncertainty as to the adequacy of a glomerular circulation should occur.

SUMMARY

In a series of 89 experiments on Necturi extending over $2\frac{1}{2}$ years the percentage of cases in which the molecular concentration of the glomerular fluid was the same as that of the serum, as compared by Barger's method, was distinctly correlated with the degree of adequacy of the precautions taken to prevent evaporation of fluid, approaching 100 per cent as the technique approached the ideal. Evidence is offered that the earlier findings of Schmitt and White on inorganic P content of glomerular fluid were due to a dilution of fluid from an external source. Glomerular function is very probably a passive filtration process.

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FURTHER CONSIDERATIONS OF THE PROPERTIES OF THE GONAD-STIMULATING PRINCIPLE OF MARE SERUM

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Previous publications on the gonad-stimulating principle of mare serum have dealt primarily with its effect upon the immature female rat and upon its chemical properties.[1] In this paper particular attention will be given to the effect upon the male. In addition, further data will be presented on the effect upon the immature female and on the stability of the substance.

EFFECT UPON MALE RATS.[2] *Immature males.* Smith and Engle (1927) by means of pituitary implants were able to increase the size of the accessory reproductive organs of immature male rats. However, they found very little effect upon the testes. Later it was shown that hypophysectomy resulted in an atrophy not only of the accessory reproductive organs of the male, but also of the testes (Smith, 1930). He also found that a very remarkable regeneration of the testis occurred following hypophyseal implants in these animals. There was a restoration of spermatogenesis and also an increase in the number of interstitial cells following the implants. Moore and Price (1931) were not able to demonstrate any great effect upon spermatogenesis when hypophyses were implanted into immature male rats.

Table 1 gives the weights of the reproductive organs of injected immature males as compared to littermate controls. These rats were autopsied four days after the first injection. It will be seen that the testes are approximately doubled in size as a result of the injection, whereas there is a still greater response of the seminal vesicles and prostate glands. These rats were given 500 to 1500 rat units. The actual amount of serum injected varied from 5 to 15 cc. In the next section dealing with the reaction in the immature female rat we describe our method of standardization of the gonad-stimulating hormone in mare serum.

[1] Papers dealing with this subject have appeared as follows: This Journal, xciii, no. 1; xciv, no. 3; Anat. Rec., xlix, no. 3, and Endocrinol., xv, no. 3.

[2] A preliminary report upon the effect of the gonad-stimulating hormone of mare serum on male rats was published in the abstracts of papers presented at the annual meeting of the American Dairy Science Association, July, 1931.

In studying the histology of the testes of these injected animals, we found more conspicuous changes in the tubules than in the interstitial cells. The tubules were greatly enlarged, as compared to the controls, and the number of spermatocytes was increased (figs. 1 and 2). There was a slight increase of the interstitial tissue following injection, but never as conspicuous as that shown by Moore and Price (1931). Their periods of treatment were more extended than were ours.

The seminal vesicles of injected animals were sometimes six times as heavy as those of littermate controls. Some of this increase in weight is due to accumulation of secretory products within the gland and this probably explains why the seminal vesicles of immature rats show a more marked response as regards weight than the prostate.

TABLE 1

The effect of the gonad-stimulating hormone of mare serum upon the genitalia of male rats

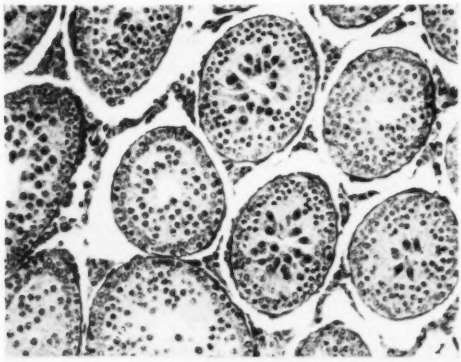
DESCRIPTION OF RATS USED	RAT UNITS ADMINISTERED	AGE AT AUTOPSY	NUMBER OF RATS USED	AVERAGE WEIGHTS			
				Body	Testes	Seminal vesicles	Prostate
		days		*grams*	*grams*	*grams*	*grams*
Immature males on normal diet	None	26–29	7	55	0.263	0.007	0.053
	500–1,500	26–29	7	55	0.492	0.023	0.124
Mature males on normal diet	None	100–131	14	440	3.788	1.104	1.221
	500–1,500	100–131	16	448	3.854	1.403	1.534
Mature males on low-protein diet	None	125–320	7	208	2.427	0.219	0.296
	500–1,500	125–320	5	202	2.555	0.614	0.797

Mature males on normal diet. By referring to table 1, it will be observed that the injection of the gonad-stimulating hormone of mare serum did not produce any conspicuous changes in the reproductive organs of mature males. Also, a histological study of the reproductive organs did not reveal outstanding changes. Perhaps a more prolonged treatment would have been more efficacious. Although the results we obtained were not clear-cut, slight changes of the same nature as those occurring in the reproductive organs of the injected immature males did occur. There is a possibility that greater changes were produced in the seminal vesicles and prostate than we were able to measure. Ejaculation and the consequent emptying of the seminal vesicles may have partially masked our results.

We have also observed hyperemia of the gonads of both sexes after

administration of the gonad-stimulating hormone of mare serum. This was conspicuous in the mature males even though the increase in weight of the testes was not marked (fig. 3).

Mature males on low-protein diet. Guilbert and Goss (1932) in their study of the effects of restricted protein intake on the oestrous cycle and



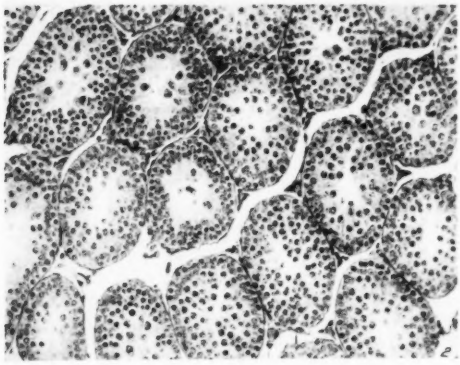


Fig. 1. Photomicrograph of testis of rat B 2688 injected with a single dose of 500 R. U. of the gonad-stimulating hormone of mare serum and autopsied four days later at 29 days of age. × 125.

Fig. 2. Photomicrograph of testis of rat B 2690, littermate control for B 2688, autopsied at 29 days of age. × 125.

gestation in rats found that diets containing 3.5 to 5 per cent protein but otherwise adequate resulted either in cessation of oestrum or in long and irregular cycles. It was also found that male rats, after subsisting for some time on one of these low-protein diets refused to mate when placed with normal females which were in oestrum.

These males were allowed to develop on a normal diet until 50 to 60 days of age, at which time they were changed to the low-protein diet. They usually became impotent after two or three months, during which time there was a significant loss in weight. Examination of the epididymii of males which had become impotent revealed motile sperm. This suggested the possibility that failure to breed might be due to atrophy of the accessory reproductive organs.

Blood serum of pregnant mares was injected subcutaneously into 9 of these impotent males. These males had been tested with normal females before injection with the serum. The number of tests preceding the injection varied from 4 to 12 and only one positive mating was obtained. The

Fig. 3. Reproductive organs of male rats autopsied at 130 days of age: a—rat W 2371 on low protein diet; b—rat G 2392 on low protein diet receiving a single dose of 500 R. U. of gonad-stimulating hormone of mare serum; c—rat G 2394 on normal diet; d—rat B 2395 on normal diet receiving 500 R. U. of gonad-stimulating hormone of mare serum.

usual dose of serum was 5 cc. (500 R. U.). Positive matings were obtained from all but one male on the third or fourth day following injection. The effect of the serum apparently continued for some time since as many as 7 positive matings were secured from individuals tested regularly over a period of 15 days. Out of a total of 30 positive matings 15 were fertile, 14 of which resulted in normal young. One male mated four times during a period of 15 days following injection and all of these matings resulted in litters. This individual mated 4 additional times at irregular intervals during a period extending from 22 to 60 days after injection. None of these later matings was fertile. With another male six positive matings were secured during a 12-day period following injection, none of which

proved fertile, the females in most instances coming into oestrum after the normal interval. In most of these cases of infertile matings, a very small number of sperm was found in the vaginal smears. Some of these males were in extremely poor physical condition.

In addition to the study of the sexual activity of these males on the low-protein diet, we also studied the effect of the injection upon the weight (table 1) and the histology of the reproductive organs. The increase in size of the seminal vesicles and prostate was conspicuous in every instance and was comparable to the increase secured in the immature rats. However, changes in the weight of the testes were smaller and somewhat inconsistent. The weight of the seminal vesicles of one animal was seven times that of its littermate control. This increase in weight is partly due to the increased amount of seminal fluid. Figure 3 gives some indication of the response which may be elicited in these low-protein rats.

The fact that blood serum, rich in the gonad-stimulating principle, induced oestrum in females which had ceased ovulating, and induced sexual activity in males which had become impotent, suggested that the interference with the reproductive process in these rats fed protein deficient diets might be related to hypo-function of the hypophysis.

In order to test further the question of whether the reproductive failure of these rats on a low-protein diet involved the hypophysis, we implanted the glands from some of them into immature females and compared the reaction to that obtained from the glands of littermate controls fed on a normal diet (table 2). A vaginal reaction was obtained in all cases. With one exception, the increase in the weight of the ovaries in those females which received glands from the normal males was greatly in excess of that attained by those receiving the glands from the low-protein males. In the instance where the ovarian response from the hypophyses of low protein animals was equal to that of the controls (recipient B 3044), one individual was included among the donors which had been repeatedly injected with potent serum in the course of breeding tests which were conducted some time prior to autsopy. Whether this accounts for the variance from the rather consistent results obtained from the other six tests is a matter of conjecture.

If rat B 3044 be omitted the average weight of the ovaries of the rats receiving the glands from the controls was about $2\frac{1}{2}$ times that of those receiving glands from the low-protein animals. However, the weight of glands implanted from the controls was nearly double that of the low-protein rats. That the weight of the hypophysis decreased in the rats on the low-protein diet is indicated by the fact that the average hypophysis weight of these rats was 5.1 mgm., while those from 8 normal males autopsied at the same age and weight attained by the low-protein group before being placed on the restricted diet was 8.3 mgm. The weight of hypophysis

per 100 mm. of length (body plus tail) for the low-protein rats was 1.28 mgm. while that for normally-fed males averaged about 2.0 mgm. per 100 mm. of body-tail length.

That the hypophysis is not permanently injured has been indicated by the rapidity with which the normal oestrous cycle is resumed when females on low-protein diets are given adequate nutrition. In some preliminary attempts to determine the amount of gonad-stimulating hormone in the hypophyses of low-protein animals we ground them in saline and injected them into immature females. The response was much poorer than that of

TABLE 2

Implantation of hypophyses of male rats on low-protein and normal diets into immature females

Five glands were implanted into each recipient

DESIGNATION OF RECIPIENT	WEIGHT OF GLANDS IMPLANTED	WEIGHT OF OVARIES OF RECIPIENT*	AVERAGE BODY WEIGHT OF DONORS	AVERAGE LENGTH OF DONORS	DIET OF DONORS
	mgm.	*mgm.*	*grams*	*cm.*	
B3060	27.6	20	202	40.3	Low protein
B3061	27.0	18	230	40.3	
B3054	23.3	18	201	39.4	
B3055	30.0	15	234	41.3	
B3040	21.2	30	204	39.0	
B3044	24.2	79	206	39.8	
G3048	27.2	20	217	40.3	
W3057	39.0	60	406	44.2	Normal
W3058	44.3	70	508	45.6	
W3059	41.3	60	495	45.9	
GH3039	53.3	80	509	45.5	
B3056	49.0	80	503	46.7	
B3042	51.0	78	468	45.5	
GH3045	51.0	60	473	45.8	

* All of the recipients showed a vaginal smear of oestrum on day of autopsy.

the implant method, indicating that it is necessary to use the intact gland to receive the maximal response. We do not feel that the amount of gonad-stimulating hormone present in the gland gives a true indication of its activity. There is some question as to how our results should be interpreted. Perhaps the difference in weight between the hypophyses of the low-protein rats and their littermate controls is more significant than the difference in the reactions of the glands when implanted into immature females.

EFFECT UPON IMMATURE FEMALE RATS. *The assay of the gonad-stimulating hormone of mare serum.* We have found the immature female rat

to be the most convenient test animal for the assay of the gonad-stimulating hormone in mare serum. Before discussing the factors influencing the reaction we will present our method of assaying the hormone. We use rats 25 days old on the day of injection and autopsy them five days later at 30 days of age. If one has no data on the concentration of the hormone it will be necessary to run a preliminary test of the serum to determine the

TABLE 3

The assay of the gonad-stimulating hormone of mare serum

The rats were 25 days old when injected and were autopsied 5 days later at 30 days of age.

DESIGNATION OF RAT	DOSE ADMINISTERED	WEIGHT OF OVARIES	NUMBER OF MATURE FOLLICLES OR CORPORA	VAGINAL REACTION	EXPRESSION OF DOSE IN RAT UNITS
	cc.	*mgm.*			
W3352	0.01	17	None	−	0.5
G3362		14	None	+	
GH3368		17	None	−	
W564a		14	None	−	
W3374		20	None	−	
GH3375		26	8	+	
Average		18.0	1.3		
G3353	0.02	22	6	+	1.0
G3363		22	5	+	
GH3369		22	7	+	
W565a		12	None	+	
BH3376		20	6	+	
BH3377		22	8	+	
Average		20	5.3		
B3355	0.04	24	6	+	2.0
G3364		27	8	+	
G3370		40	7	+	
B566a		20	8	+	
B567a		21	8	+	
G3378		33	8	+	
Average		27.5	7.5		

approximate concentration. In general it is only practicable to use serum in which the hormone is highly concentrated and, therefore, if 0.04 cc. of the serum does not produce a reaction we discard it. If, however, 0.04 cc. or less will produce the reaction, one can usually determine with fair accuracy the concentration of the serum by injecting 4 groups of rats, consisting of six rats to a group, with 0.005, 0.01, 0.02 and 0.04 cc. respec-

tively. The result of such a test is shown in table 3. The group receiving 0.005 cc. was omitted from the table since all the rats in the group were negative. Our definition of a rat unit is as follows: *A rat unit of the gonad-stimulating hormone of mare serum is the amount which will produce, in a group of six rats, an average of from three to ten mature follicles or corpora for each immature female rat tested and autopsied five days after the injection and half of which amount will fail to consistently produce a vaginal smear of oestrum in another group of six rats.* We will give our reasons for so defining the rat unit of the hormone in question. To begin with, we feel that the vaginal smear should be considered in defining a rat unit as it is at present the most sensitive means for detecting small amounts of the hormone. In the test previously referred to (table 3) 0.01 cc. produced a vaginal smear of oestrum in G3362 although no change took place in the ovaries which could be detected macroscopically. The same was true for W565a receiving 0.02 cc. Thus, if one-half the amount necessary to produce an average of from three to ten corpora or mature follicles fails to produce a vaginal smear of oestrum in all of six rats, one is assured that the end point is being approached at which ovarian changes will be produced. As the vaginal smear changes are determined by oestrin it is evident that the ovary of the immature rat must also be considered. When lower levels of the hormone are given the change in ovary weight as compared to control ovaries is small. For example, the average weight of the two ovaries in a group of 18 control rats autopsied at 30 days of age was 17 mgm. The average weight of the two ovaries in the group receiving 0.02 cc. was 20 mgm. (table 3). Thus it is evident that large numbers would be necessary if weight were used in place of number of mature follicles or corpora.

Factors affecting the reaction. In an effort to determine the factors affecting the character of the gonad-stimulating hormone reaction, we have compared the reaction produced by repeated as compared to single dosages, by various sized dosages, and by rats of varying ages. Table 4 gives the results obtained by repeated as compared to single dosages in 25-day old rats as measured by ovarian weight. It will be noted that the minimal amount which will produce sexual maturity when given in single doses is insufficient to do so when distributed over a 4-day period. Larger amounts given in single or in 8 doses produced similar reactions.

Sixty-four rats were used to compare the reaction in the ovaries of rats 21, 25 and 28 days old at the time of injection. These rats were autopsied 6 days later in order to allow sufficient time to elapse for ovulation to take place. Since this experiment was completed we have found that rats may be autopsied on the fifth day following the injection, that is, if they have not ovulated on the fifth day ovulation will not take place until a later heat period. No significant differences dependent upon age were observed

in the size of the dose necessary to bring on sexual maturity. The ovaries of the older rats were larger than those of the younger rats receiving similar amounts of hormone, but the character of the reaction was very much the same irrespective of age.

TABLE 4

The effect of repeated dosage as compared to single dosage of the gonad-stimulating hormone upon the weight of the ovaries of immature rats

Three rats were used in each group. The rats were 25-days old at the time of the first injection and were autopsied at the 31st day of age

NUMBER OF RAT UNITS ADMINISTERED	AVERAGE WEIGHT OF BOTH OVARIES OF RATS RECEIVING 8 DOSES IN 4 DAYS	AVERAGE WEIGHT OF BOTH OVARIES OF RATS RECEIVING SINGLE DOSE
	mgm.	*mgm.*
1	19*	25
5	32	38
10	34	32
50	95	124
100	172	174

* Ovaries of all rats in this group were infantile.

TABLE 5

The number of ovulations resulting from the injection of varying size dosages of the gonad-stimulating hormone into rats 21, 25 and 28 days of age on the day of injection

The rats were autopsied 6 days after the injection.

NUMBER OF RAT UNITS ADMINISTERED	21 DAY OLD RATS		25 DAY OLD RATS		28 DAY OLD RATS	
	Number of rats used	Number of rats ovulating	Number of rats used	Number of rats ovulating	Number of rats used	Number of rats ovulating
1	2	1	3	0	2	1
5	2	1	3	1	3	1
10	3	1	3	0	3	1
50	2	0	3	0	3	0
100	2	0	2	0	2	1
1,000	1	0	3	0	2	0

Table 5 presents the ovulation data for 44 of the 64 rats. With doses of 10 rat units or less, approximately one-third of the rats ovulated. Inasmuch as the number of ovulations in the 21-day old rat group was approximately the same as for the 28-day old rat group it is impossible to say that age, within the limits used in this experiment, is a factor regarding ovulation. The oviducts were sectioned serially and examined for ova. All of the ova found were in a state of degeneration, indicating that ovulation had taken place some time before. Engle (1931) has assembled the data

on the relationship between first oestrum and ovulation in the rat and mouse and states that ovulation is coincident with the first oestrum in less than one-half of the instances. Therefore, the number of ovulations resulting from the injection of the gonad-stimulating principle of mare serum approaches that of the first normal oestrum. This is also comparable to the data presented by Engle (1931) in regard to the number of immature mice ovulating following anterior pituitary implants.

It is difficult to determine from the literature the percentage of animals ovulating following the injection of urine of pregnant women or extracts of the urine. Engle (1929) was unable to produce ovulation in the mouse by injection of urine of pregnant women. Although Zondek (1930) has written voluminously on the reaction of the urine of pregnancy in the immature rat and mouse we can find no data which he gives on this point further than the statement that the injection of "Prolan A" under special conditions will produce ovulation. He does not state what these special conditions are. One can only conclude from the data at hand that the injection of urine of pregnancy rarely produces ovulation in the immature rat or mouse.

In regard to the factors affecting the character of the ovarian reaction following the injection of the gonad-stimulating hormone of mare serum, the only one which is clear-cut is the size of dose. Large doses uniformly bring about the mass production of corpora lutea atretica without ovulation. Ovulation occurs when doses up to ten times the minimal dose are given. The data which we have do not support the view that repeated doses are more effective than single dosage. Further, we were surprised to find that the age of the rat within the limits of our experiment was not a factor in regard to the number of rats ovulating. The number of animals in each group is small and it is possible that larger numbers might show some differences in this regard.[3]

[3] We have some data on the number of rat units necessary to produce an ovarian response in other species. Cole and Miller (unpublished data) produced ovulation in 13 out of 15 ewes injected during anoestrum with 50 to 500 R. U. of the gonad-stimulating hormone of mare serum. Lower doses produced very little, if any, change in the ovaries and higher doses resulted in the production of cystic follicles with only an occasional ovulation. Thirteen non-injected controls did not ovulate. A curious feature of this experiment was that none of these ewes bred although tested repeatedly with rams known to be potent during the breeding season. We now have experiments in progress to determine as to whether this failure to breed was due to the impotency of the rams. We feel that it is unlikely that ovulation was produced in the ewes unaccompanied by heat. It appears from our data that 1000 rat units are sufficient to produce oestrum in young sows. As an example the following experiment is presented: Four sows in a litter of eight were given a single injection of 1000, 2000, 3000, and 6000 rat units respectively. The remaining four sows were left as controls. All four of the injected sows showed evidence of heat on the 4th and 5th day following the injection. Three of them were bred, became pregnant, and two of the three gave birth to litters. The third sow of those which became pregnant died of pneu-

DATA ON THE STABILITY OF THE HORMONE. The rats used in the following experiments were twenty-five days old when injected and were autopsied on the fifth day thereafter at 30 days of age. Ovaries of 18 control non-injected rats at 30 days of age had an average weight of 17 mgm. with extremes of 11 and 21 mgm. The average body weight of this group was 78 grams with extremes of 63 and 90 grams. We now regularly autopsy our rats on the fifth day instead of on the fourth in order to check more accurately the number of rats in which ovulation is produced. Only one injection was made excepting in the instances in which 10 cc. were injected and in those instances 5 cc. daily on two consecutive days were injected. The ovarian weights recorded in the table have reference to the weight of the ovaries with oviducts and bursae removed. In all instances the amount injected is stated in terms of original serum. The serum to be used was tested for potency coincidently with the test of the treated serum.

Effect of storage at 1°–3°C. One of the important characteristics of the gonad-stimulating hormone found in the serum of pregnant mares is its relative stability. We have stored sera in ice cream cartons for approximately two years during which time so much moisture had been lost that the residue was solid and brittle. Upon diluting this solid residue with Locke's solution we found the hormone still present. For example, 27 grams of this residue were diluted with 100 cc. of Locke's solution and then injected. One-tenth cubic centimeter of this dissolved material produced sexual maturity and ovarian development in immature rats. Large doses (1 cc., 5 cc. and 10 cc.) produced ovarian development beyond that produced by the serum when first drawn. Similar results were obtained from retesting a sample of serum after storage in a sealed flask for 200 days. This, and other data, indicates that although the amount to produce a minimal reaction remains fairly constant or increases slightly after long standing, larger doses of the serum after standing produce larger ovaries than similar size doses of fresh serum. Possibly some inhibiting factor is slowly destroyed upon standing.

Effect of oxidation. The above data indicate that the active principle

monia during the course of pregnancy. The fourth sow, which did not become pregnant, was probably bred. She received the largest dose and from other autopsy data which we have on young sows injected with the gonad-stimulating hormone it is likely that this large dose resulted in the production of a large number of corpora lutea atretica. This experiment is not critical, however, as one of the four controls came into heat five days after the injected sows, was bred, and became pregnant. The three remaining controls did not come into oestrum until several weeks later. Other experiments with sows 120–150 days of age would indicate that doses similar to those used in the above experiment will produce oestrum in four to seven days. In a preliminary experiment six cows were given doses of 5000 to 12,000 rat units. They were killed six days later but, unfortunately, were not tested for oestrum. Three of them were given intravenous and three subcutaneous injections. The ovaries were enlarged in all instances, some of them as large as a medium-sized orange.

is not easily oxidized. However, we have studied this point further. Seventy cubic centimeters of serum were shaken for seven hours at room temperature in a 6 liter round-bottom flask in a continuous current of air. The control serum was allowed to stand in a closed flask at room temperature for an equivalent length of time (table 6). Aeration had very little if any effect upon the hormone during this seven-hour period.

TABLE 6

The stability of the gonad-stimulating hormone of mare serum when subjected to oxidation, action of acid, alkali and pepsin

TREATMENT OF SERUM	AVERAGE WEIGHT OF OVARIES RESULTING FROM INJECTION OF:				
	0.01 cc.	0.02 cc.	0.1 cc.	0.5 cc.	5.0 cc.
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Untreated (control)		31	40	159	291
Aerated		28	64	125	265
Iodine		23	29	82	208
Untreated (control)		49	50	149	209
Acid pH 5		27	102	140*	180*
Acid pH 4		34	39	260*	210*
Acid pH 3		60	65	150	264*
Acid pH 2		32†	80	125	262
Untreated (control)	35		48	136	188
Pepsin	17‡	26	26	63	251
Untreated (control)		24	25	118	190
Alkali pH 8		22	30	81	186
Alkali pH 9		28	32	63	258
Alkali pH 10		17	22	39	255

* One rat only used in the test. All other ovarian weights represent the average of 2 rats.

† No large follicle or corpora in the ovaries of either of the rats in this group.

‡ Ovaries appeared infantile in both instances but one showed a reaction in uterus and vagina, indicating that some ovarian development had taken place as it takes 30 cc. of this serum to produce a vaginal reaction in a spayed rat.

Next, we treated serum with iodine. The serum was made N/100 with respect to iodine. Only a portion of the iodine was reduced during the half-hour of exposure preceding injection into immature female rats. This treatment did reduce the potency slightly (table 6). The results demonstrate, nevertheless, the stability of the hormone to mild oxidizing agents.[4]

[4] We have not studied the effect of bacterial action upon the hormone. When it is desired to keep serum for future use it is our procedure to pass the serum through a Berkefeld filter and store it in sterile containers.

Treatment of serum with acid. In previous experiments results obtained by treating serum with pepsin and trypsin were inconsistent (Goss and Cole, 1931). Therefore, we decided to study the effect of acid separately. Five composites of a single sample were taken; one remained untreated except for dilution, the other four were brought to a pH of 5, 4, 3, and 2 respectively with N/3 HCl. All samples were then incubated overnight at 38°C. After incubation the treated samples were brought to a pH of 7.4 with sodium hydroxide. The results appear in table 6. No apparent effect upon the potency resulted until a pH of 2 was reached. One one-hundredth cubic centimeter of serum at pH 2 failed to produce sexual maturity or ovarian development. Thus it is apparent that the pH at which pepsin works would not be particularly injurious to the hormone.

Treatment with pepsin. Inasmuch as acid failed to destroy the hormone, we reinvestigated the effect of pepsin. One per cent of pepsin powder was added to a sample of serum which had been adjusted to pH 3 with N/3 HCl. The sample was incubated overnight at 38°C. After incubation the pH was adjusted to 7.4 and the serum injected. The pepsin powder was previously tested with egg white and found to be highly active.

As may be seen in table 6 there is some loss of potency (approximately one-half). A portion of the serum treated with pepsin was dialyzed. The hormone remained in the sac with the residue as in the case with untreated serum.

Effect of alkali. In a previous paper (Goss and Cole, 1931) we showed that the feeding of large amounts of the hormone was without effect, indicating either that the hormone was not absorbed or that it was destroyed. As we have proved that acid alone or pepsin working in an acid media does not appreciably destroy the hormone, we next studied the effect of alkali (table 6). Possibly some destruction resulted but it was not marked as is indicated by the fact that there was ovarian development in all groups injected with 0.02 cc.

SUMMARY

Immature male rats injected with the gonad-stimulating hormone of mare serum show marked responses in the seminal vesicles and prostate. Also, the testes are approximately doubled in size. The tubules of the testes are enlarged and there is an increase in the amount of interstitial tissue. Mature males on a normal diet do not show marked changes in the reproductive organs following injection although there is evidence of increased secretory activity in the prostate and seminal vesicles. Males on a protein deficient diet show responses in the prostate and seminal vesicles comparable to immature rats but the response of the testes is less conspicuous. Impotency in these low-protein rats is temporarily cured by the injection of the hormone.

Consideration is given to the factors affecting the reaction in the immature female rat. A method of assay of the hormone using immature female rats is suggested. The hormone will produce ovulation when given in small doses. Larger doses result in the production of large numbers of corpora lutea atretica. Data are appended regarding the reaction of the females of other species to the hormone.

The hormone as it occurs in serum is relatively stable in acid and alkali (pH 2 to pH 10) and is not easily destroyed by oxidation or by pepsin.

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ON THE INTERACTION OF OESTRIN AND THE OVARY-STIMULATING PRINCIPLES OF EXTRACTS OF THE URINE OF PREGNANCY

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The problem of the antagonism and interaction of sex hormones has been studied quite extensively during recent years. Experiments proving conclusively that the ovary acts as an inhibitor of the effects of the anterior lobe secretions, as well as studies on the functions of oestrin and the corpus luteum hormone in this inhibition, have been carried out. The problem of direct hormonal interaction, however, has not been studied quantitatively heretofore. This is the subject of our experiments.

Evans and Simpson (4), (5), (6) find that hypophyses of gonadectomized animals are much more potent in their ovary-stimulating power than those of normal animals. Engle (2), (3) and Fluhman and Kulchar (7) report identical findings. Meyer, Leonard, Hisaw and Martin (12), (13), studied the effects of continuous injections of oestrin on the stimulating power of the hypophysis of normal and of castrated rats. With the normals they find that the ovaries of recipients of hypophyses from injected animals weigh 40 per cent less than those receiving implants from controls, and with castrates that the ovaries of recipients from injected animals weigh 28 per cent less than ovaries of recipients from castrate controls. They also tested the effect of continuous injection of oestrin into immature rats and find that ovaries of injected animals weigh 40 per cent less than those of controls. Golding and Ramirez (8) report similar results. Kunde, D'Amour, Spencer, Gustavson, and Carlson (10), (11), (16) studied the effect of continuous injection of oestrin on the various organs of the mouse. They find 1, that ovaries of the injected animals weigh 43 per cent as much as those of controls, and 2, that the development of the ovaries is much less in injected animals; no developing follicles are found; 3, a smaller anterior lobe is noted, and hyperplasia of the thyroid.

Doisy, Curtis and Collier (1) studied continuous injections of oestrin, and injection of a massive dose, into immature rats, finding that those receiving a massive dose are always about 10 days behind the normals in weight and development of the generative organs; and that the total growth

of the ovaries of those receiving continuous injections is $\frac{1}{16}$ that of the normal growth at the end of four weeks.

These experiments show a depressive action of the ovary on the hypophysis, and point to oestrin as the depressing agent. Moore (14), (15) demonstrates a similar effect of oestrin on the male hypophysis.

One of the points remaining to be cleared up is the question whether there is direct interaction of the hypophyseal hormones and of oestrin. This point is studied in experiment I by making simultaneous injections of oestrin and the hypophysis hormones into ovariectomized mice.

Mice of the Bagg albino strain, inbred since 1913, were used between the ages of $1\frac{1}{2}$ to 3 months. Absolute uniformity of test animals was assured, as the remotest relationship is that of second cousin.

These mice were ovariectomized in sets of 15 to 20 at a time, and the actual experiment was started 15 days after operation. This arbitrary time was so chosen because the oestrous cycle shown by all ovariectomized mice directly after the operation is certainly completed at that time.

The hormones used were follutein,[1] a preparation of the hypophyseal hormones obtained by extraction from the urine of pregnant women both the luteinizing and the oestrogenic principles, the latter according to our tests being present in excess, and theelin[2] (oestrin). In one experiment Antuitrin S, a Parke-Davis preparation containing an excess of the luteinizing principle, was used.

The procedure was as follows. Each set of animals was again divided into two sets, 8 to 10 animals per set. These animals were injected on the fifteenth day after operation with simultaneous injections of varying doses of hypophysis hormone and of oestrin. Injections were made dorsally, subcutaneously, and the two hormones were injected in different portions of the back so that no mingling of the hormones could take place under the skin. The hormone preparations were made up by diluting standard preparations with sterile Ringer-Locke solution to the correct concentration, so that $\frac{1}{10}$ cc. of the diluted substance equaled the dosage which the animal was to receive.

If one of these two sets of animals received, for example, an injection of 3 mouse units (m. u.) of follutein, to 1 m. u. of oestrin, the other received 6 m. u.:1 m. u. of oestrin. Vaginal smears were taken from 8 to 12 days. The animals were then all injected with a dose of oestrin equivalent to the one they had received in the first series of injections (in this case, 1 m. u. of oestrin). Vaginal smears were followed again for 8 to 12 days. Then the dosages in the simultaneous injections were reversed, so that the

[1] Through the kindness of Dr. J. F. Anderson of E. R. Squibb & Sons, we received a regular supply of assayed fresh hormone.

[2] We are indebted to Dr. E. A. Sharp of Parke, Davis & Co. for the Parke-Davis preparations used in these experiments.

animals receiving 3 m. u. of follutein in the first series, now received 6 m. u., whereas those which had received 6, now got 3 m. u. of follutein, again to 1 m. u. of oestrin. Vaginal smears were continued for about 20 days to detect whether regeneration of ovarian tissue had taken place. Those animals not showing regeneration were then used in other preliminary experiments.

This procedure is of great advantage, in that every test animal is its own check. The vaginal smears obtained after oestrin injection are used as a basis of comparison for those smears obtained after simultaneous injections of the two hormones, and it is easy to see whether inhibition of oestrin action has occurred. (The hypophysis hormone alone, of course, has no effect, since no ovaries are present to be stimulated.) It is apparent that a positive inhibition in one animal, in which the oestrin action alone was quite strong, might not be called an inhibition in another animal which did not respond as well to oestrin injection.

One disadvantage of this technique is that since the entire dosage of one hormone is given in one injection, not all the hormone will be absorbed and a portion is excreted without any effect on the organs. However, this disadvantage is met by the fact that every test animal is injected under the same conditions, and that those fractions of the dosage which are absorbed are relatively comparable.

Vaginal smears were taken at regular hours each day so that the interval between two smears was always 24 ± 2 hours. Thus again all smears were made comparable. The first smear was taken 22 ± 2 hours after injection.

The types of inhibition of oestrin action are classified under three headings—total, partial, slight, with a fourth heading including those animals showing no inhibition. The classification is made on the basis of intensity, duration, and delay in appearance of cornification.

Regeneration of ovarian tissue takes place in a large percentage (51 per cent) of the animals. In the case where in spite of regeneration there is an inhibition, there is no need to exclude the animal from the tables; on the contrary the effect is doubly certain, since the follutein neutralized both the injected oestrin and the small amount of oestrin it caused to be secreted. Where, however, no inhibition is gotten and directly afterwards regeneration is evidenced, the animal may be excluded from our tables, because the neutralization may have taken place, but may have been masked by the effect of the oestrin secreted by the fragments of regenerated ovarian tissue. The criterion of regeneration is a cyclical appearance of cornification beginning 10 days after the last injection.

The following two contingency tables—table 1, in which regeneration was not considered, table 2, in which it was taken into consideration, show the distribution in the various dosages, and in the groups of three headings

of low dosage (1 and 2 m. u. follutein vs. 1 m. u. oestrin); medium (3, 4, 5 and 6 m. u. follutein vs. 1 m. u. oestrin); and high dosage (8 and 10 m. u. follutein vs. 1 m. u. oestrin).

TABLE 1

Regeneration not considered

OESTRIN: FOLLUTEIN ↓ DOSAGE	INHIBITION				
	Total	Partial	Slight	None	Total
1:1	0	0	2	14	16
1:2	0	1	1	14	16
1:3	5	2	2	4	13
1:4	6	5	0	4	15
1:5	10	6	7	6	29
1:6	3	2	7	1	13
1:8	4	1	3	7	15
1:10	5	5	1	6	17
	33	22	23	56	134

	INHIBITION				
	Total	Partial	Slight	None	Total
Low (1,2:1) dosages:					
Observed	0	1	3	28	32
Expected	7.87	5.25	5.49	13.37	
Medium (3,4,5,6:1) dosages:					
Observed	24	15	16	15	70
Expected	17.24	11.34	12.01	29.25	
High (8,10) dosages:					
Observed	9	6	4	13	32
Expected	7.87	5.25	5.49	13.37	
Total	33	22	23	56	134

The lower figure in each cell represents the mathematical expectation, defined by Pearson as the product of the total of the column with the total of the row, divided by the total number of observations.

Table 1 leads to the following conclusions: 1, low dosages. Significantly less inhibition than the mathematical expectation. Correspondingly more than expected in the "none" column. This shows that 1 and 2 m. u. of follutein are not sufficient to suppress the oestrin action. 2, medium dosages. Significantly more inhibition than the mathematical expectation. Correspondingly less than expected in the fourth column. There is defi-

nite inhibition in this range of dosage. 3, high dosages. Very little deviation from the expected values. Interpreted in comparison to the other two rows, this means, that there is definitely more inhibition than in the low, and less than in the medium dosages.

In table 2, a still more sharp division, with the same tendencies as in the preceding table, is seen. The results are more clear-cut, showing relatively more inhibition in the medium dosages.

TABLE 2

Regeneration considered

OESTRIN: FOLLUTEIN ↓ DOSAGE	INHIBITION				
	Total	Partial	Slight	None	Total
1:1	0	0	2	12	14
1:2	0	1	1	10	12
1:3	5	2	1	1	9
1:4	6	5	0	1	12
1:5	10	6	6	3	25
1:6	3	2	5	0	10
1:8	4	1	3	6	14
1:10	5	5	1	5	16
	33	22	19	38	112

	INHIBITION				
	Total	Partial	Slight	None	Total
Low (1,2:1) dosages	0 7.66	1 5.11	3 4.41	22 8.82	26
Medium (3,4,5,6:1) dosages	24 16.50	15 11.00	12 9.50	5 19.09	56
High (8,10:1) dosages	9 8.84	6 5.89	4 5.09	11 10.18	30
Total	33	22	19	38	112

The percentage inhibition, i.e., the percentage of the first three columns of the total number of observations is as follows:

1. Not taking regeneration into consideration.....57 5 per cent
2. Excluding mice showing regeneration from the last two columns.....65.2 per cent
3. Not including low dosages, or sub-threshold values.....81.4 per cent

We can conclude, therefore, that interaction has taken place in the test animals.

If neutralization occurs in vitro—in a simple chemical reaction—animals injected with mixtures of the hormones should show more inhibition than those injected separately, since the hormones have been in contact longer. The following experiment indicates that this is probably not the case.

	TOTAL	PARTIAL	SLIGHT	NONE	TOTAL
1 m.u. oestrin *plus* 4 m.u. follutein	2	2	3	3	10

The distribution does not differ radically from that in the foregoing tables.

One m. u. of oestrin is shown to be the maximal dose inhibited by 20 or less m. u. of follutein, in the following experiment.

	TOTAL	PARTIAL	SLIGHT	NONE	TOTAL
8 m. u. follutein: 2 m. u. oestrin	0	0	0	6	6
10 m.u. F.: 2 m.u. O	0	0	1	8	9
20 m.u. F.: 2 m.u. O	0	1	0	8	9
	0	1	1	22	24

The reason for this is probably due to the fact that in the case of 2 m. u., the absorption of oestrin is too rapid to permit neutralization. In the following experiment the 2 m. u. are given as follows: 1 m. u. with the follutein, the other 5 to 7 hours later. Had this interval been longer, we would probably have gotten more definite results.

	TOTAL	PARTIAL	SLIGHT	NONE	TOTAL
10 m.u. F.: 1 plus 1 m.u. O. (7 hrs. apart)	0	2	3	4	9
20 m.u. F.: 1 plus 1 m.u. O (5 hrs. apart)	0	0	2	7	9
	0	2	5	11	18

Another series of injections was made using antuitrin which contains more luteinizing and less oestrogenic hormone. The results cannot be interpreted, first, because there are too few cases, and second, because the relative proportions of oestrogenic and luteinizing hormones in the two preparations used are not so well established that definite conclusions can be drawn.

	TOTAL	PARTIAL	SLIGHT	NONE	TOTAL
4 m.u. antuitrin: 1 m.u. oestrin	2	0	2	5	9

Apparently similar experiments with strongly luteinizing extracts of pregnancy urine have been made by De Jongh and Dingemanse (9). Their results were negative. Simultaneous injections of their extract and small amounts of oestrin into immature rats resulted in the suppression of oestrus manifestations, due apparently to ovarian luteinization. Interaction of oestrin and the urine extracts may have occurred, however.

In a second experiment the effect of simultaneous injections of oestrin and follutein into immature mice was studied. The object was to find the dosage of oestrin which would suppress a dose of follutein known to cause good follicular development and ovulation; or, if that was not the case, to observe by histological methods the effect of oestrin *plus* follutein on the ovary, as compared with the effect of the same dose of follutein.

An assay of follutein revealed that a dosage of 4 m. u. given in one injection was sufficient to cause definite follicular development in 100 hours. In the antagonism injections (20 and 10 m. u. of oestrin : 4 m. u. follutein) using 5 and 8 animals respectively, it was noticed that follicular development was just as strong if not stronger than in the assay controls. However, there seemed to be less luteinization than in the controls. It appears that 20 m. u. of oestrin are not sufficient to cause suppression of the effects on the ovary of 4 m. u. of follutein. Unfortunately, the effect of higher dosages of oestrin on the same dose of follutein is not available for discussion.

SUMMARY

1. It was observed that oestrin is directly antagonistic to follutein when both are injected into ovariectomized mice.

2. A similar effect is had when the ovary-stimulating hormone is antuitrin S.

3. No definite suppression was had of Follutein effects on the ovaries of immature mice when 10 or 20 m. u. of oestrin were injected simultaneously with the follutein (4 m. u.).

4. A technique for studying hormonic antagonism and interaction is presented. The general method is as follows: Extirpation of the gland studied. Study of effects of removal. Replacement therapy by injection of hormone of that gland. Then simultaneous injection of other hormones to see which of them suppresses or enhances the replacement effect of the first hormone.

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THE EFFECT OF STIMULATION OF THE CERVICAL SYMPATHETIC TRUNK UPON THE ENERGY METABOLISM OF RABBITS

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The experiments to be described in this paper were designed to study the changes in energy metabolism following stimulation of the nerve supply of the thyroid gland. Anatomists agree that the gland receives nerve fibers from the cervical sympathetic trunk, and Nonidez (1931) has recently given evidence that in the dog the upper portion of the nerve is the source of most of the fibers to the gland. The question as to whether these fibers exert only a vasomotor influence on the gland or in addition to this a secretory influence is not settled. Mason, Markowitz and Mann (1930) by means of a plethysmographic study have shown that the cervical sympathetic sends vasoconstrictor fibers to the gland. With regard to a secretory nerve supply the evidence is not nearly so convincing. The results of studies of the histological changes following section of the nerve have been conflicting. Thus Misseroli (1909) found characteristic changes in the granules of the secreting cells and Wiener (1909) found a marked reduction in size as a result of section of the cervical sympathetic trunk. Crawford and Hartley (1925), on the other hand, found no histological changes occurring as a result of either section or stimulation of the cervical sympathetic or the vagus and its branches. Evidence gained from a study of differences in iodine content of the two lobes following stimulation of the cervical sympathetic trunk on one side seems to support the idea of a nervous control of the gland cells (Watts, 1915, and Rahe, Rogers, Fawcett and Beebe, 1914).

Early physiological evidence for a nervous control of thyroid activity was given by Asher and Flack (1911) who gave evidence that stimulation of the laryngeal nerves acted like injection of thyroid extract in that both caused an increase in the effect of the depressor nerve upon blood pressure. These workers found also an increased effect of adrenalin in raising blood pressure under the same conditions. Cannon, Binger and Fitz (1914) found in cats a rise of as much as 125 per cent in the rate of energy metabolism following phrenic-cervical sympathetic anastomosis. These results were interpreted as being due to a continuous stimulation of the gland by

impulses originating in the respiratory center. This experiment failed to yield positive results in the hands of Burget (1917), and Marine, Rogoff and Stewart (1917). Electrical changes occurring in the thyroid as a result of cervical sympathetic stimulation were used as evidence for a nervous control of the gland by Cannon and Cattell (1916). Cannon and Smith (1922) demonstrated a 25 per cent increase in rate of the denervated heart as a result of either massage of the thryoid or stimulation of the cervical sympathetic trunk. Neither anemia of the gland nor stimulation of other nerves gave this effect. They concluded that the rise in heart rate was due to increased liberation of thyroid hormone. Rogoff (1918) collected blood from the thyroid vein of dogs before and after stimulation of the thyroid nerves and applied the tadpole feeding test. His results were negative practically. Hektoen, Carlson and Schulhof (1927) demonstrated by means of a precipitin test that there was no increase in the amount of thyroglobulin in the thyroid vein blood as a result of cervical sympathetic nerve stimulation, direct thyroid massage, or intravenous injections of adrenalin or pilocarpine.

Since the rate of energy metabolism is a fairly reliable index of the state of activity of the thyroid, and the effects of the thyroid hormone are long lasting, it would seem that experiments in which animals were observed for many days following sympathetic stimulation might offer information in regard to the problem of thyroid innervation.

Apparatus and method. A modified Haldane open circuit apparatus was used for all of the metabolism tests. The weighings were made on a large balance sensitive to about 20 mgm. The apparatus and method of computation were those described by Marine (1922).

Forty-eight rabbits have been used in this series of experiments, and about six hundred metabolism tests have been made. In order to establish individual normal rates of metabolism each animal had one to five tests before beginning an experiment. In all cases the animals were taken off feed twelve to twenty hours before each test. The operations were carried out under aseptic precautions, and as a result very few animals had to be discarded because of infection. In some cases very light chloral hydrate-urethane anesthesia was used while in others ether was given during the dissection, and no anesthetic during the stimulation. No apparent variation in results could be traced to difference in method of anesthesia.

The experiments carried out may be divided into four groups. In group 1, twenty-six rabbits were used. The cervical sympathetic trunk was exposed on both sides, cut low in the neck, and the cephalic end stimulated with an interrupted tetanic current over a period varying from one to three hours. In order to be sure that impulses were being carried over the nerve as a result of the stimulation, the rabbits' ears were arranged over a light in such a way that vasoconstriction could be readily observed with each period of stimulation.

In group 2 five rabbits were used. Thyroidectomy was performed and the metabolic rate followed until it reached a low hypothyroid level. Stimulation was then carried out as in group 1.

In group 3 six rabbits were used as controls. The sympathetic trunk was cut low in the neck as in group 1, but no stimulation was applied.

In group 4, five rabbits were used. Stimulation was carried out as in group 1, and immediately after, thyroidectomy was performed.

The forty-two rabbits in the above groups and six others have been used in establishing normal metabolism.

Metabolism tests were made in some cases daily and in others every other day for the first ten days after operation, and following this every two to five days over a period, in many cases as long as forty to eighty days.

RESULTS AND DISCUSSION. The rate of metabolism in normal adult rabbits has been found by previous workers (Pommerenke, Haney and Meek, 1930) to average 2.61 calories per kilo per hour. In the present research, the results of tests on forty-eight normal rabbits averaged 2.62 which is in close agreement with the previous series. The figure 2.62 calories per kilo hour is therefore, I believe, a reasonably accurate one for the average rate of energy metabolism in the rabbit. In certain cases, however, animals will be found which show consistently a normal rate of metabolism which varies considerably from the average figure. Because of this fact the data in the accompanying tables are presented not only in percentage variation from 2.62 but also in average percentage variation from the individual normal rates of metabolism.

In group 1 in which stimulation of the cervical sympathetic was carried out, the average maximal variation above the individual normal rate of metabolism, without regard to time of occurrence, was 35 per cent. Twenty-one animals showed a maximal rise of 20 per cent or over, fourteen a rise of 30 per cent or over, nine a rise of 40 per cent or over, six a rise of 50 per cent or over, and five a rise of 60 per cent or over. In nineteen of the twenty-six animals used this maximum occurred between the second and eighth days following stimulation. Typically the rate began rising on the second day. In twelve of the twenty-one rabbits showing a maximal rise of 20 per cent or over, the metabolic rate was followed until it fell to within 12 per cent of the normal. This occurred in five cases between thirty-one and sixty days; in three between sixteen and thirty days; in one at sixty-five days; and in three at five days or less. The rise in rate in the case of the latter three being so much shorter than the others may reasonably be referred to other sources than the thyroid. The results on the animals in group 1 have been summarized in table 1 with regard to the number of days following stimulation. This obscures the high maximal variations already mentioned, but gives a better view of the experiments as a whole. According to this table the rate of metabolism begins rising significantly

on the second day following stimulation, reaches a maximum at eleven to fifteen days, and falls to within 10 per cent of the normal at forty-one to sixty days. Since the rate of metabolism rises markedly and remains at a high level for a number of days, I believe the evidence is very much in favor of the view that there has been an increased activity of the thyroid due to sympathetic stimulation.

To show that the presence of the thyroid gland is necessary in the production of the rise in metabolism, the cervical sympathetic trunk was stimulated in thyroidectomized animals and the rate of metabolism followed. In five such animals (group 2) which showed an average metabolic rate of 1.71 Cal. per kilo per hour or 35 per cent below the normal figure,

TABLE 1

Metabolism of 26 rabbits following stimulation of the cervical sympathetic trunk (group 1)

DAYS FOLLOWING STIMULATION	NUMBER OF ANIMALS	NUMBER OF TESTS	AVERAGE CALORIES PER KILO PER HOUR	PERCENTAGE VARIATION FROM 2.62*	AVERAGE PERCENTAGE VARIATION FROM INDIVIDUAL NORMAL
				per cent	*per cent*
1	19	19	2.71	+3.4	+7.4
2	16	16	3.04	+16.0	+19.0
3	20	20	3.17	+21.0	+21.0
4	19	19	2.97	+13.4	+18.0
5	16	16	3.09	+18.0	+22.0
6–10	23	54	3.06	+16.8	+20.0
11–15	15	29	3.27	+24.7	+29.0
16–20	13	21	3.05	+16.7	+23.0
21–30	13	36	3.15	+20.3	+23.0
31–40	12	29	2.97	+13.4	+17.0
41–60	9	38	2.74	+4.3	+9.6
61–90	5	20	2.53	−3.4	+1.6

* Average of 135 tests on 48 normal rabbits.

stimulation of the cervical sympathetic was performed. During the first two days following stimulation the metabolic rate rose to 17.5 per cent above the previous hypothyroid level. On the third day, however, the rate had fallen to within 5 per cent of this level, and during the succeeding days it did not rise above that figure. Table 2 (a) shows the effect of stimulation of the cervical sympathetic trunk in these thyroidectomized animals. The results seem to indicate that the presence of the thyroid gland is essential to the long-lasting rise in metabolic rate which occurs following cervical sympathetic nerve stimulation.

The question of the involvement in our experiments of factors other than the nervous effect on the gland in the production of the rise in metabolic rate has been investigated as follows. Section of the cervical sympathetic

trunk was performed in six animals (group 3) and no stimulation was applied. It was thought that the effect of any increase in blood flow through the gland as a result of removal of the vasoconstrictor nerve supply might thus be determined. The animals in this group showed an average maximal variation above the individual normal, without regard to time of occurrence, of 14 per cent. Four of the six rabbits in this control group never varied more than 15 per cent above their individual normal figures.

TABLE 2

DAYS FOLLOWING (a) STIMULATION (b) CUTTING	NUMBER OF ANIMALS	NUMBER OF TESTS	AVERAGE CALORIES PER KILO PER HOUR	PERCENTAGE VARIATION FROM (a) 1.71 (b) 2.62	AVERAGE PERCENTAGE VARIATION FROM INDIVIDUAL PERVIOUS RATE
(a) Metabolism of 5 rabbits which had been rendered hypothyroid by thyroid removal and then subjected to cervical sympathetic stimulation. The average metabolic rate before stimulation was 1.71 calories per kilo per hour (group 2)					
				per cent	*per cent*
1	5	5	1.92	+12.3	+13.8
2	4	4	2.01	+17.5	+18.0
3	4	4	1.80	+5.2	+5.0
4	3	3	1.75	+2.3	+5.0
5	3	3	1.73	+1.2	−1.0
6–10	5	10	1.70	−0.6	0.0
11–15	3	4	1.54	−9.9	+8.0
(b) Metabolism of 6 rabbits in which the sympathetic trunk was cut low in the neck but no stimulation applied (group 3)					
1	4	4	2.60	−0.8	+3.0
2	5	5	2.63	−0.4	+2.0
3	3	3	2.69	+2.7	+8.0
4	2	2	2.80	+6.8	+13.0
5	4	4	2.71	+3.4	+8.0
6–10	6	16	2.55	−2.7	0.0
11–15	4	6	2.63	−0.4	+4.0
16–20	6	7	2.65	+1.1	+3.0
21–30	5	9	2.56	−2.3	0.0
31–40	5	7	2.60	−0.8	+1.0

The results of this group are tabulated according to post-operative days in table 2 (b). Since in this control group the average maximal variation above the individual normal was only 14 per cent while in group 1 it was 35 per cent, it is readily seen that any increase in blood flow through the thyroid has not been entirely responsible for the rise in energy metabolism following severance and stimulation of the nerve. Although vasomotor effects are not hereby ruled out, the evidence becomes stronger for a secretory activity of the sympathetic on the thyroid.

A graphic representation of the average variations from individual normal rates of metabolism in groups 1, 2 and 3 is given in figure 1.

The next question investigated was whether the rise in metabolic rate is due to liberation of thyroid hormone at the time of stimulation, or to liberation at a later time from a gland made hyperactive as a result of the stimulation. Plummer and Boothby (1921) showed that the greatest rise in metabolic rate occurred on the eighth day following a single intravenous

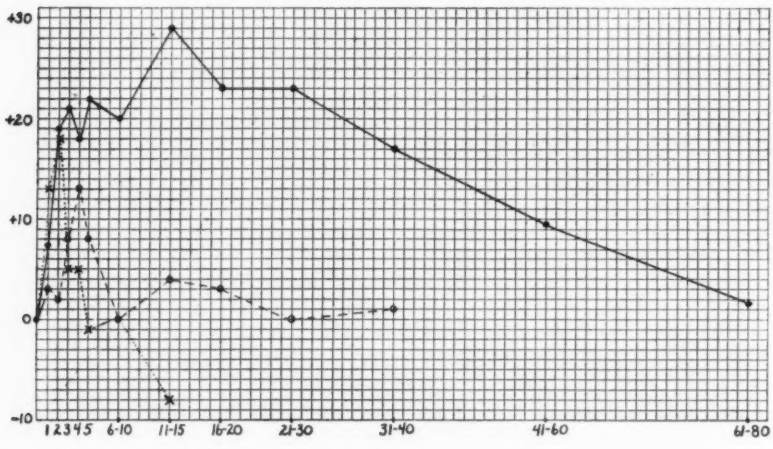

Fig. 1. Abscissae—Days following stimulation.

Ordinates—Average percentage variation from individual normal rates of metabolism except in group 2 as described below.

●——●—The 26 rabbits which were subjected to cervical sympathetic section and stimulation (group 1).

×-----×—The 5 rabbits which were subjected to thyroidectomy and allowed to develop a low metabolic rate, and then cervical sympathetic stimulation applied (group 2). The curve represents average percentage variation from the individual hypothyroid levels.

○.... ○—The 6 rabbits which were subjected to cervical sympathetic section only (group 3).

dose of thyroxin in clinical cases. Later Boothby and Sandiford (1924) showed that the effect may last forty-eight days. These results would seem to indicate that the changes in metabolic rate reported in the present research may be due to liberation of thyroid hormone at the time of nerve stimulation. Kunde (1927), however, showed that as soon as seven to twelve hours following single intravenous injections of thyroxin in dogs, the metabolic rate rose markedly, and then fell to normal within three to five days. Her results would seem to indicate that following sympathetic

stimulation, as in our experiments, a longer-lasting mechanism is at work. In order to present evidence on this point, five rabbits were subjected to sympathetic stimulation and immediately following to thyroidectomy (group 4). The results are tabulated in table 3 (a), and can readily be compared with the results of thyroidectomy alone as given in table 3 (b). It will be seen that even though thyroidectomy immediately followed the

TABLE 3

(*a*) *Metabolism of 5 rabbits whose cervical sympathetic trunks were stimulated, and thyroid glands removed immediately after (group 4)*

DAYS FOLLOWING STIMULATION AND THYROIDECTOMY	NUMBER OF ANIMALS	NUMBER OF TESTS	AVERAGE CALORIES PER KILO PER HOUR	PERCENTAGE VARIATION FROM 2.62	AVERAGE PERCENTAGE VARIATION FROM INDIVIDUAL NORMAL
				per cent	*per cent*
1	4	4	3.24	+23.7	+12 0
2	3	3	2.95	+12.6	+7.0
3	5	5	2.61	−0.4	−8.0
4	5	5	2.88	+9.9	+1.0
5	5	5	2.60	−0.8	−8.0
6–10	5	11	2.35	−10.3	−18.0
11–15	5	9	2.31	−11.8	−19.0
16–20	5	7	1.96	−25 2	−31 0

(*b*) *Metabolism of 9 thyroidectomized rabbits (no stimulation)*

DAYS FOLLOWING THYROIDECTOMY	NUMBER OF ANIMALS	NUMBER OF TESTS	AVERAGE CALORIES PER KILO PER HOUR	PERCENTAGE VARIATION FROM 2 62	AVERAGE PERCENTAGE VARIATION FROM INDIVIDUAL NORMAL
				per cent	*per cent*
1	4	4	2.56	−2 5	+2 0
2	3	3	2.43	−7 2	+3 0
3	2	2	2.30	−12 0	−14 0
4	5	5	2.41	−8.0	−1 0
5	7	7	2.14	−18.3	−10 0
6–10	6	13	1.93	−26 3	−19 0
11–15	8	12	1.73	−34 0	−27 0
16–20	8	10	1.81	−30.9	−24 0
21–30	6	12	1.72	−34 3	−27 0
31–40	3	5	1.88	−28 2	−20 0
41–60	1	3	1.84	−29.7	−29 0

nerve stimulation, the average rate of metabolism during the first two days is distinctly higher than in the case of the animals thyroidectomized without any previous nerve stimulation. It would seem, therefore, that at least a portion of the rise in metabolic rate may be referred to liberation of the hormone at the time of nerve stimulation. As early as the fifth day following operation, however, those animals which were stimulated before

thyroidectomy showed as low an average percentage variation from the individual normal rate of metabolism as those thyroidectomized without previous stimulation. On the other hand, the animals in group 1 gave an average rate on the fifth day after stimulation of 22 per cent above the normal. These facts indicate that the high metabolic rate found in the latter group is not entirely due to increased liberation of hormone at the time of stimulation, but is due partly to an over-active gland which was brought up to increased activity as a result of the nerve stimulation. The logical conclusion is that stimulation does exert a secretory effect upon the gland cells, this effect being manifest for a considerable time following stimulation.

CONCLUSIONS

1. Stimulation of the cervical sympathetic trunk in twenty-six rabbits resulted in a marked rise in the rate of energy metabolism beginning at the second day, reaching a maximum of 29 per cent above normal at eleven to fifteen days, and returning to normal in forty-one to sixty days.

2. The thyroid gland is essential to the above effects for, in thyroidectomized rabbits subjected to cervical sympathetic stimulation, the rate of energy metabolism does not rise significantly above the hypothyroid level.

3. The increased blood flow through the gland which may be assumed to occur as a result of the cutting of the vasoconstrictor nerve fibers in the cervical sympathetic trunk, does not seem to be the cause of the increase in rate of energy metabolism. Six rabbits failed to show a significant rise following cutting of the nerve, without subsequent stimulation.

4. At least a portion of the increased liberation of thyroid hormone may occur at the time of stimulation. In five rabbits in which thyroidectomy immediately followed sympathetic stimulation, a significant rise in energy metabolism occurred before the fall to thyroidectomy level.

5. Following thyroidectomy of rabbits the energy metabolism begins falling on the fifth post-operative day and continues to fall to an average level of around 30 per cent below normal which is reached in about two weeks.

6. The evidence from our experiments indicates that stimulation of the cervical sympathetic does result in a secretory effect upon the thyroid gland. The rate of energy metabolism in rabbits subjected to this stimulation is 22 per cent above the normal at five days, while the rate in animals subjected to thyroidectomy immediately after stimulation is at the same level at five days as those animals thyroidectomized without any previous stimulation.

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THE EFFECT OF MUSCULAR WORK AND COMPETITION ON GASTRIC ACIDITY

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During the century since Beaumont's classical experiments on Alexis St. Martin but few observations on man have been added to those from which he drew his inference that moderate exercise facilitates gastric digestion. In 1846 Combe expressed the dual belief that "rest of body and tranquillity of mind for a short time, both before and after eating, are necessary and conducive to healthy digestion." Cannon's work (1925) demonstrated experimentally that certain emotional states such as unpleasant feelings, anxiety, anger or fear may delay secretion and persist in their effect long after the removal of the exciting condition. In 1928 Campbell, Mitchell and Powell studied a series of normal young men given, before exercise, a test meal of bread, meat and potatoes, or a modification of the Boas meal of oatmeal gruel. The severity of the exercise to which these young men were subjected was roughly measured and consisted usually of running around the laboratory, the distance varying from one to four miles. They concluded that such exercise delayed digestion but lighter exercise, walking, had no inhibitory influence upon gastric secretion. In its application to the field of athletics, interest centers not only on the muscular exertion itself, but also on the emotional stress coincident to competition, as influences possibly modifying gastric secretion. The purpose of this study is to add to the data concerning the influence of exercise upon the gastric secretion of man, and to observe the effect upon gastric function of muscular exertion accompanied by emotional excitement.

RESTING GASTRIC ACIDITY. The first series of observations was made on M. M. M., a normal, healthy subject accustomed to vigorous exercise, a young woman of 21 with professional training in physical education. Ewald's test-breakfast was administered 14 to 18 hours after the last meal. A Rehfuss tube was swallowed and the stomach was aspirated one hour subsequent to the beginning of the test meal. The filtrate was titrated and examined for free and total acidity. After the subject had been trained to swallow the stomach tube, a series of ten observations was made to establish the normal resting acidity values following the test-breakfast. The results are expressed in terms of the number of cubic centimeters of

decinormal sodium hydroxide necessary for the neutralization of 100 cc. of gastric juice, each cubic centimeter representing one degree of acidity. Total acidity averaged 47.6° and HCl 29°, 60.92 per cent of the total acidity being due to free hydrochloric acid. From a review of records of gastric analysis made at the Mayo Clinic and from a study of data in the literature, Vanzant et al. (1932) selected a series of 3746 cases for the establishment of age group standards of normal gastric acidity. She and her associates found that the modal free acidity for women is approximately 35 units throughout adult life with a normal range of about 90 units, modal total acidity being practically constant at 51° between the ages of 20 and 60. Subject M. M. M.'s normal resting acidity falls within the limits of these standards.

EXERCISE FOLLOWING THE TEST MEAL. The subject came to the laboratory and in place of resting for the hour between the beginning and the aspiration of the test meal, she rode the electrodynamic brake bicycle ergometer. The muscular exertion was at first equivalent to 336.44 kgm.m./min. at an average pedalling rate of 63 revolutions per minute. It was repeated on ten different days, increasing in severity to 489.36 kgm.m./min. with the training of the subject. Immediately after the termination of the exercise the gastric contents were aspirated and analyzed. Total acidity was moderately reduced, averaging 39.4°. The depressing influence was more marked as regards hydrochloric acid. It dropped by 33 per cent to an average value of 19.3°, but 48.98 per cent of the total acidity of the post-exercise gastric juice being due to free HCl. A separate analysis of the first and last five records reveals that notwithstanding an augmentation in the rate of working, the latter demonstrated a less marked average decrease in free and total acidity, and three individual records surpassed the normal standard. The increase in the severity of the work did not balance the effects of training, and as the subject became more proficient in this exercise it had a decreasing inhibitory influence upon gastric acidity, eventually augmenting both free and total acidity to a level above the resting normal. These findings are demonstrated in figure 1.

EXERCISE BEFORE THE TEST MEAL. Four experiments were performed with 60 minutes of physical exertion preceding the administration of the test meal. The rate of working averaged 550 kgm.m./min. at a mean pedalling rate of 68 revolutions per minute. Immediately after the termination of the exercise the meal was eaten and the subject remained quietly seated until the usual period of aspiration. Total and free acidity both increased moderately, reaching 53.25° and 35.75° respectively, 67.13 per cent of the total acidity now being due to free HCl.

A single observation was made after an exhausting bout of work. Before coming to the laboratory the subject spent six hours in vigorous, outdoor exercise. The usual breakfast was eaten but luncheon was substi-

tuted by 500 cc. of special Guernsey milk and the juice of 8 oranges taken in small quantities at short intervals preceding midday. No food was taken for 7 hours prior to the experimental exercise. The subject then did a severe and exhausting piece of work on the ergometer, riding for 60 minutes, the average rate of working being 703.46 kgm.m./min. The test meal was administered after the cessation of exercise and aspirated in one hour. Both total acidity and free hydrochloric acid were markedly sup-

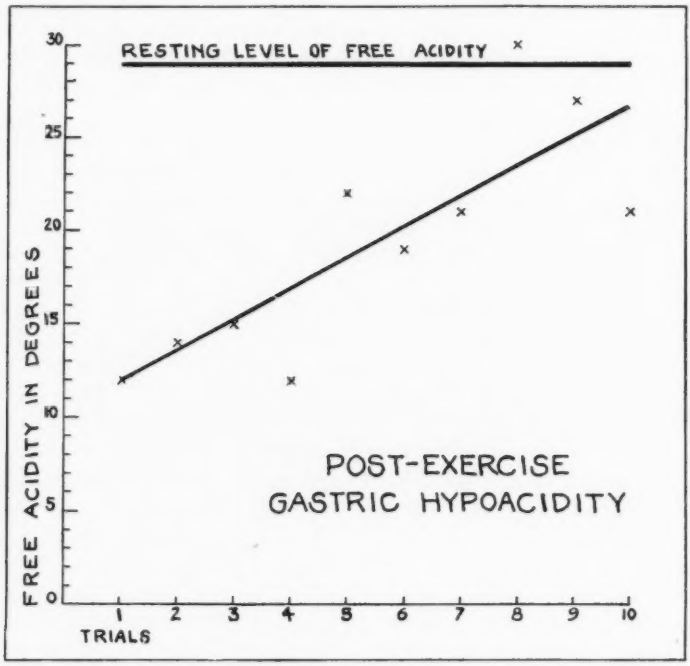

Fig. 1. Ten successive experiments showing the decreasing influence of exercise following a test meal, upon free gastric acidity. Observed HCl in cubic centimeters of N/10 NaOH necessary to neutralize 100 cc. of gastric juice and straight line fitted by least squares.

pressed, occurring in 29° and 6° in the order designated, but 20.68 per cent of the total acidity being due to free HCl.

Figure 2 is a composite of these 25 experiments. It shows that exercise during and immediately following an Ewald meal reduced both total acidity and free acid, figure 1 showing the inhibition to be less marked as the subject became trained. Exercise preceding the test meal augmented gastric acidity, except when it was exhausting, such exercise being followed by a marked diminution in the degree of acidity.

CONFIRMATORY OBSERVATIONS. The gastro-intestinal tract is variable in its response to identical stimuli in different subjects. To confirm the trend of the reactions of a single individual, three different persons were subjected to a series of controlled observations similar to the experimental procedures of M. M. M. These subjects were normal, healthy young women between the ages of 19 and 23, professional students in physical education, above the average in fitness and neuromuscular skill and accustomed to strenuous exertion.

1. *Resting gastric acidity.* The Ewald test-breakfast was administered and aspirated as described. The average of 3 observations was taken as

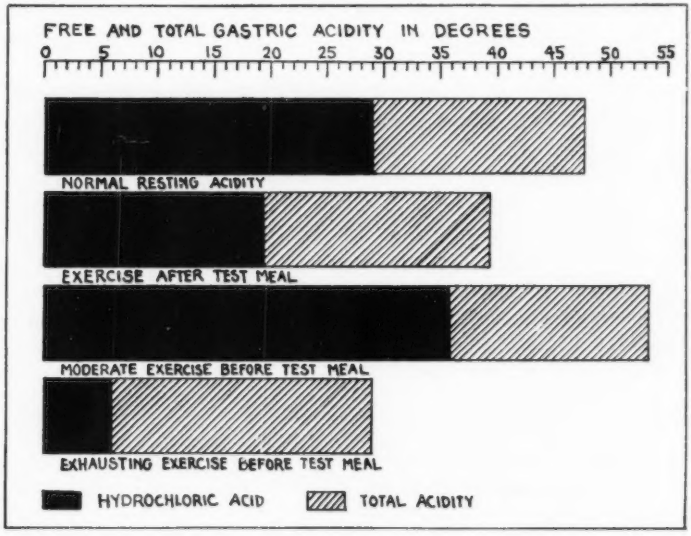

Fig. 2. Bar diagram showing the influence of exercise upon the gastric acidity of one subject.

the normal acidity for each subject. L. D. had an acidity below the modal values set by Vanzant (1932), M. M. closely approximated the standards and S. H. exceeded them. The group thus represents the norm and its extremes.

2. *Exercise following the test meal.* The subjects pedalled the bicycle ergometer for one hour doing a very moderate piece of work without any evidence of fatigue. The influence of the exercise was slight, in general stimulating, average free and total acidity being augmented by 0.4 per cent and 4.2 per cent respectively.

3. *Exercise before the test meal.* The subjects exercised for one hour

immediately before the administration of the test meal. This was the first time they had done so protracted and severe a piece of work on the bicycle ergometer and there were manifestations of fatigue, especially in subjects L. D. and S. H. who showed a post-exercise decrease in gastric acidity. Subject M. M. worked more moderately and the exercise was stimulating. On an average free acidity decreased by 7.1 per cent although there was an increase of 4.2 per cent in total acidity.

The group was subsequently subjected to gentle exercise in the endeavor to determine whether such muscular activity preceding a meal would be uniformly stimulating in the event that the exercise be sufficiently light. The three subjects took a brisk walk out of doors during the hour preceding the administration of the test meal. The results were accordant, on an average free acidity being increased by 25.94 per cent and total acidity by 20.34 per cent.

The attempt was next made to duplicate the severe and exhausting work which had had so profoundly depressing an influence upon the gastric acidity of subject M. M. M. On the day of the experiment the usual breakfast was eaten but luncheon was substituted by highly nutritious liquids. The group took a 28 mile bicycle ride, circumventing a neighboring lake and upon their return to the laboratory performed the usual 60 minute piece of exercise upon the ergostat. Subject S. H. was obviously tired. The bout of exercise was slightly depressing, free acidity decreasing by 12.75 per cent and total acidity by 0.38 per cent. On the other hand L. D. and M. M. showed no evidences of fatigue and the exercise, severe and protracted though it seemed, had a strongly stimulatory effect, free and total acidity augmenting by 81.00 per cent and 66.07 per cent respectively in M. M. and by 56.08 and 82.99 per cent in L. D.

Any exercise of endurance must of necessity be carried on in the steady state. It is known that exercise carried on at high speed incurs an oxygen debt, and the rapid accumulation of acid metabolites results in early exhaustion. The group was finally subjected to a battery of exercises carried on at high speed, the total period of exertion being limited to 5 minutes. The exercise consisted of stair climbing at top speed, rapid exercise on the stationary bicycle, violent rope jumping and rotating a Prony brake ergometer by hand. At the termination of the exercise the subjects were incoördinated, dyspneic and sweating profusely. The test meal was administered at once and aspirated in one hour. A diminution in the degree of acidity occurred in every case, the average reduction being 50.92 per cent in HCl and 33.14 per cent in total acidity.

The individual findings of this series of experiments are recorded in the accompanying tables. In trend, they confirm the observations made on subject M. M. M.

Competition. The influence of competitive sport participation upon

gastric acidity was next studied. The necessity of swallowing a stomach tube for the aspiration of gastric contents imposes from the outset a psychic disturbance which makes it difficult to obtain reliable findings under conditions not too far removed from normal. If the competition is genuine, not artificially imposed, it is difficult to obtain subjects because of

TABLE 1

Showing the average free and total acidity of the three subjects studied

SUBJECT	NORMAL GASTRIC ACIDITY		
	HCl	Total acidity	Per cent HCl
M. M.	40.33	56.00	72.01
L. D.	28.83	37.16	64.12
S. H.	65.33	86.33	75.67

TABLE 2A

Showing the influence of moderate exercise following an Ewald meal upon free and total acidity

SUBJECT	POST-EXERCISE GASTRIC ACIDITY		
	HCl	Total acidity	Per cent HCl
M. M.	44.00	60.00	73.33
L. D.	24.00	40.00	60.00
S. H.	67.00	87.00	77.01

TABLE 2B

Showing the influence of various types of exercise preceding an Ewald meal upon free and total acidity

Post-exercise gastric acidity

SEVERE EXERCISE			GENTLE EXERCISE			ENDURANCE EXERCISE			EXHAUSTING EXERCISE		
HCl	Total acidity	Per cent HCl	HCl	Total acidity	Per cent HCl	HCl	Total acidity	Per cent HCl	HCl	Total acidity	Per cent HCl
49.00	65.00	75.38	53.00	69.00	76.81	73.00	93.00	78.49	29.00	47.00	61.70
25.00	45.00	55.55	44.00	56.00	78.57	45.00	68.00	66.17	9.00	26.00	34.61
51.00	77.00	66.23	73.00	91.00	80.21	57.00	86.00	66.27	28.00	47.00	59.57

the danger of upsetting the "condition" of the players by the necessary modification of the usual pre-game procedure, especially the administration of an unpalatable meal immediately prior to the beginning of play.

The subjects were healthy, normal young women physical education students, participating in the final game of a basketball tournament, one about which many traditions center. On the day of the game the usual

breakfast was eaten. Luncheon was substituted by a high calorie liquid diet of sweetened fruit juice, egg-nog, hot chocolate and malted milk given at hourly intervals during the morning. No food was taken for 7 hours prior to the game, a test meal of dry toast and tea being administered just before the onset of play. Competition was keen and the game was fast and hard fought. At its termination, Rehfuss tubes were swallowed and the gastric contents were aspirated. The subjects had been carefully

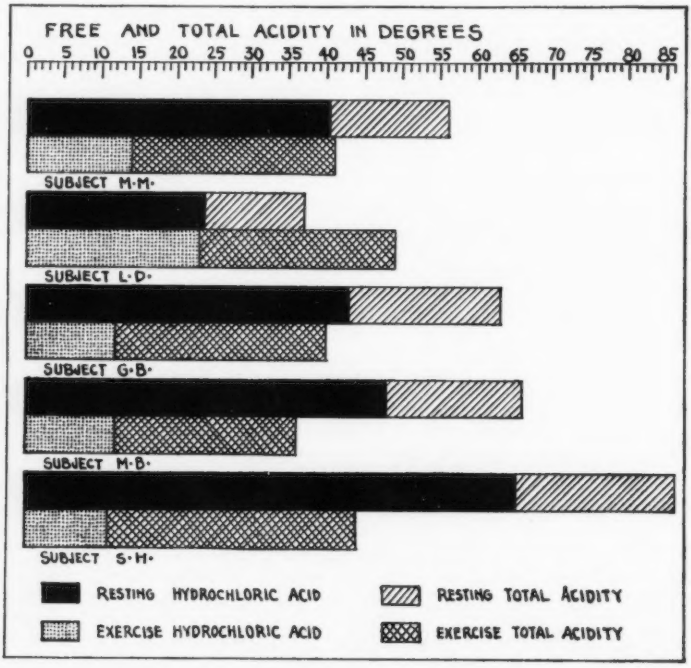

Fig. 3. Bar diagram showing the influence of competitive exercise upon the gastric acidity of five different subjects playing in the same game.

instructed concerning the technique of swallowing the tube and had witnessed the procedure. Five passed Rehfuss tubes without difficulty. The resting and exercise gastric acidities of these subjects are recorded in figure 3. Except for an increase in subject L. D. who played only during the second half of the game, there was a diminution in the total acidity of the gastric secretion. Free acidity was uniformly diminished, in general to a point far below the resting level.

Discussion. In 1927 Apperly and Semmens began to investigate the

relationship of gastric function to the chemistry of the blood. Bennett and Dodds (1921) had already noted that a high alveolar carbon dioxide was associated with high gastric acidity. Apperly with Semmens in 1928 and with Crabtree in 1931 altered the plasma bicarbonate and cH of the blood and showed that the gastric acidity varies directly with the bicarbonate content irrespective of the H ion concentration. During muscular exercise, especially when violent and carried on at high speed, lactic acid is rapidly produced, it accumulates in the blood, lowers the bicarbonate content and accelerates the output of carbon-dioxide. The lungs are unable to wash out the carbonic acid rapidly enough and the H ion concentration rises. In the light of these experimental observations the diminution in gastric acidity during violent exercise may be due to the concomitant fall in plasma bicarbonate. In accord with this hypothesis, hypoacidity was observed to appear only when the muscular exertion was associated with evidences of the incurrence of an oxygen debt and the greatest diminution in gastric acidity occurred when the exercise was severe and exhausting. The physico-chemical changes in the blood must therefore be taken into consideration in the determination of the cause of the exercise gastric hypoacidity.

If the muscular exercise is severe and generalized, splanchnic vasoconstriction diverts blood away from the visceral area to the active skeletal muscles and skin, producing a relative diminution in the oxygen supply to the stomach. This may contribute to the production of the gastric hypoacidity associated with exhaustive muscular exertion. It is common experience that psychic disturbances also modify gastric function. Apperly and Crabtree (1932) stress, in addition, the influence of emptying time upon gastric acidity. No motility observations were made in this series of studies and such may throw further light upon the interpretation of these findings.

Crandall (1928), studying the effect of physical exercise on the gastric secretion of Pavlow dogs, noted that after the cessation of exercise, the secretion may rise to a level above the normal. We observed that when gentle exercise preceded or followed the test meal, gastric acidity might exceed the resting level. Such exercise is usually unassociated with emotional disturbance and neither shunts large quantities of blood away from the visceral area nor induces marked changes in the composition of the blood. Moderate muscular exertion heightens the general metabolism, improves the circulation and has a stimulatory effect upon the organism as a whole, probably thus augmenting gastric functional activity.

SUMMARY

Gentle exercise before or after a test meal augments gastric acidity. Protracted exercise is not necessarily depressing, but exhaustive muscular

exertion, whether it precedes or follows a test meal, is associated with a diminution of the acidity of the gastric secretion to a level below resting normal, and the decrease is greatest when the exercise is accompanied by emotional excitement.

Acknowledgment. Our best thanks are due to the students who generously acted as subjects and withstood the rigors of this research.

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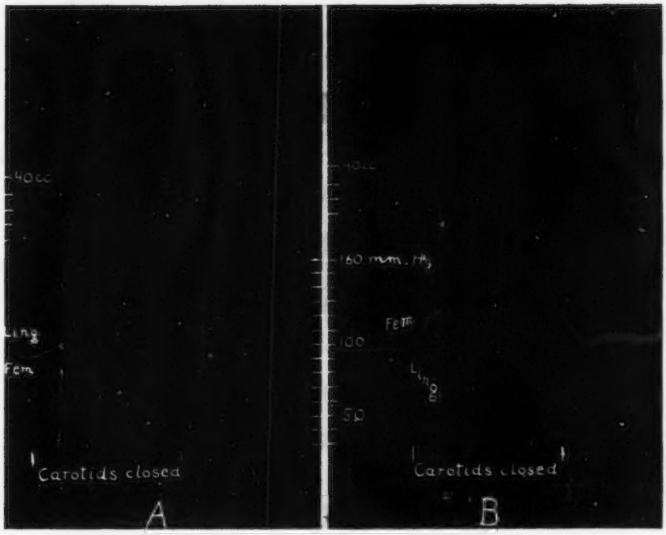

Fig. 3

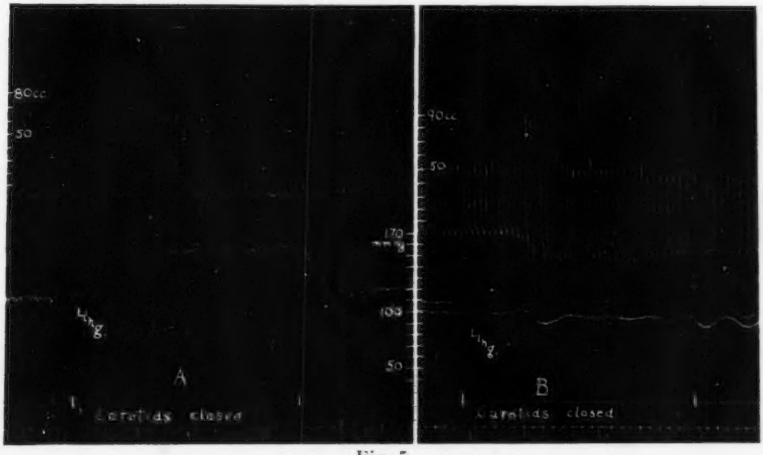

Fig. 5

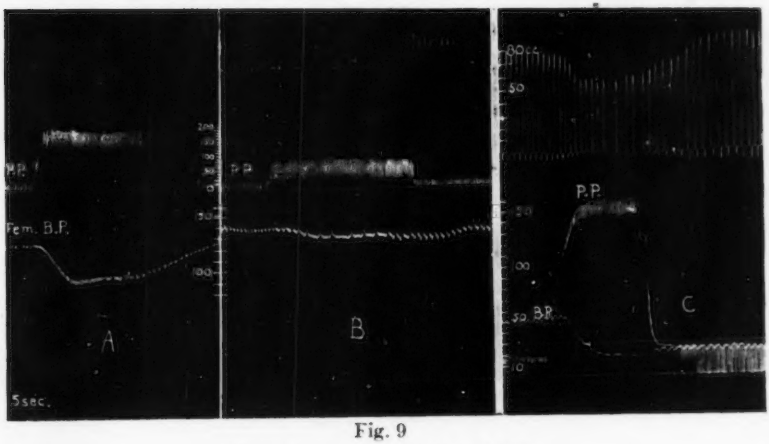

Fig. 9

(Insert between pages 118 and 119)

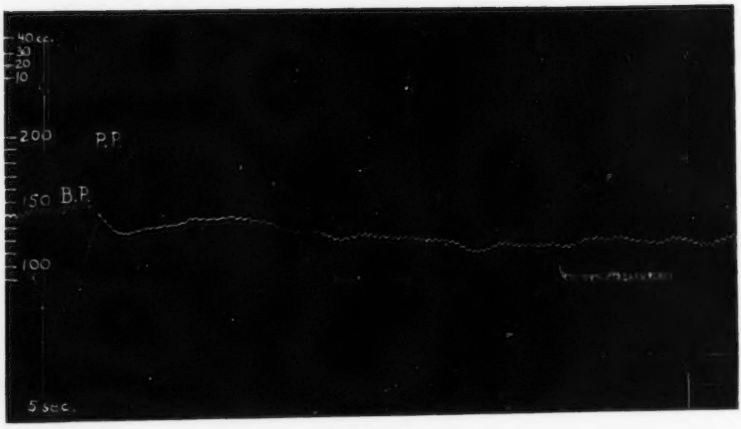

Fig. 10

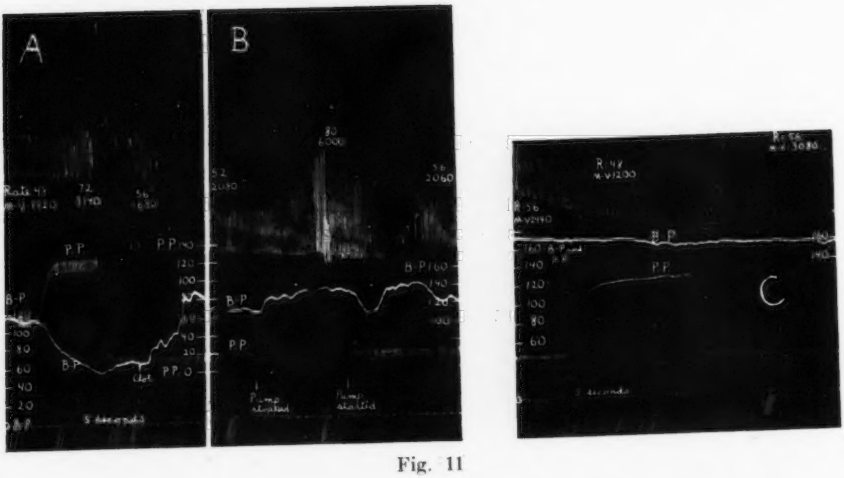

Fig. 11

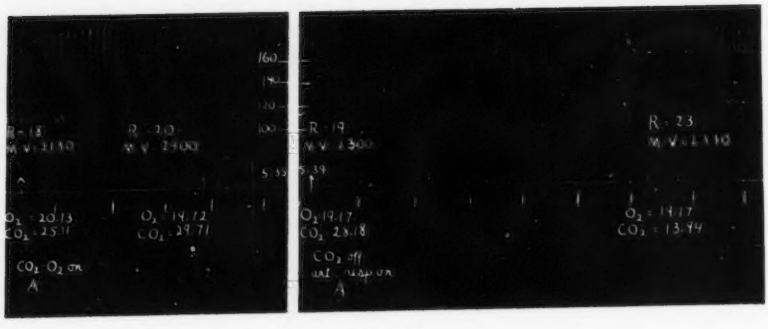

Fig. 15

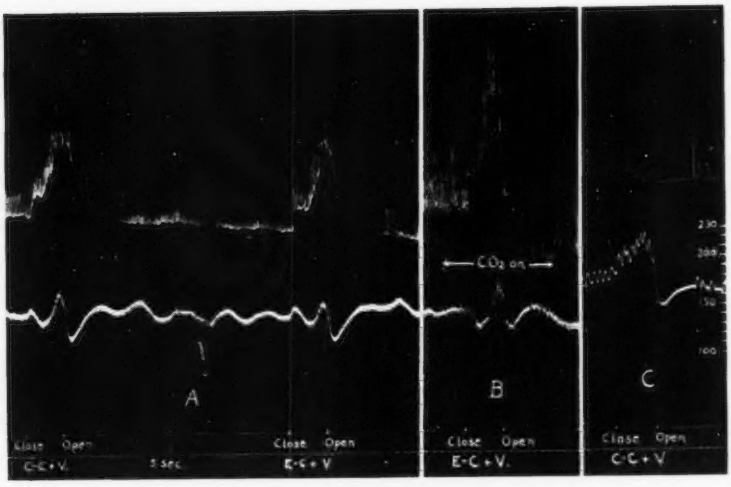

Fig. 2

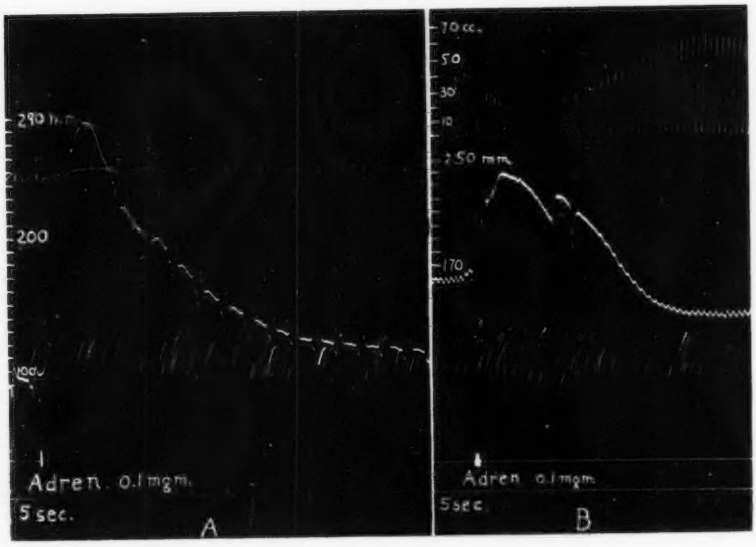

Fig. 5

(Insert between pages 136 and 137)

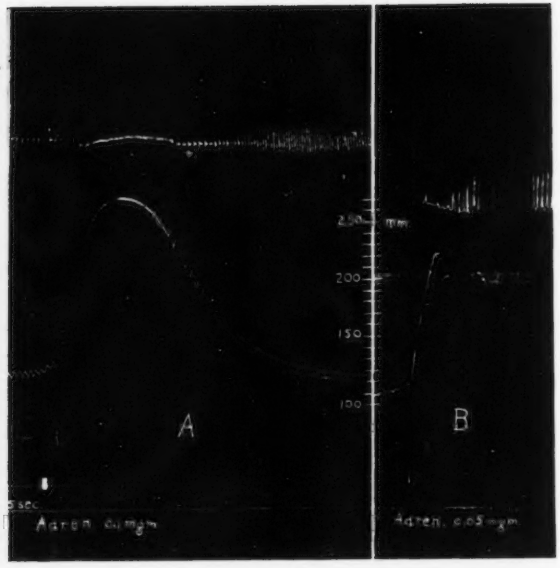

Fig. 6